The synthesis of some analogues of shikimic acid

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Award date:
1988

Awarding institution:
University of Bath

THE SYNTHESIS OF SOME ANALOGUES OF SHIKIMIC ACID

Submitted by Stephen Arthur Bowles

for the degree of PhD

of the University of Bath

1988

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To my Mother and Father

ACKNOWLEDGEMENTS

I acknowledge the support of the SERC and ICI Pharmaceuticals Division in the funding of this work.

I am sincerely grateful to Professor Malcolm M. Campbell and Dr M. Sainsbury, my supervisors at Bath, for their expert help, guidance and encouragement. Special thanks also to my industrial supervisor, Dr Gareth M. Davies, whose tireless assistance was invaluable, and much appreciated.

I wish to extend my thanks to numerous members of the Organic Chemistry Department for helpful discussions and advice.

I am indebted to many people who provided excellent technical support, not least Mrs Susan Boucher (née Green) and Mr. Richard Betteridge. Thanks to Mr. R. (Harry) Hartnell and Mr. D. Wood for n.m.r. spectra, Mr. C. Cryer for mass spectroscopy, Mr. A. Calver for microanalyses, and Dr K. Molloy and M. Mahon for their priceless contribution of X-ray crystallography. I am also obliged to the Physical Methods section at ICI Pharmaceuticals, particularly Mr. Brian Wright and Mr. David Greatbanks for several extremely useful discussions on n.m.r. spectroscopy, Mr. George Cockayne and Mr. Stuart Nicholson for their help with the kinetic work, and Mrs Madeline Vickers for mass spectroscopy on some important compounds.

Many thanks to Mrs Annie Hudson who had the unenviable task of deciphering my handwriting and typing the manuscript.

Finally, a very special thanks to my Lizzy for providing a swift kick in the appropriate place on the numerous occasions I appeared to be slacking.

ABSTRACT

The shikimic acid pathway has its origins in carbohydrate metabolism and is the principle metabolic process by which plants and lower organisms synthesise aromatic compounds, including the essential amino acids phenylalanine, tyrosine and tryptophan. It does not operate in animals, so any agent which interferes with this pathway is potentially a broad spectrum bacteriocide and fungicide which may prove effective in mammals. It is possible that analogues of shikimic acid may fulfil this function.

Described, herein, is methodology for the synthesis of 6-substituted analogues of shikimic acid, which involves extension of a previously vindicated route to a key cyclohexadiene ester intermediate, conversion of this to an epoxide, and regioselective nucleophilic epoxide cleavage.

Direct epoxidation of this diene ester with conventional peracid reagents resulted in an unexpected mixture of epoxides which proved inseparable. In contrast, epoxidation with perbenzimidic acid afforded a single, "abnormal" epoxide. Reaction mechanisms are proposed which account for these observations, and the essence of this postulate is exploited to obtain the desired epoxide exclusively.

Reaction of this epoxide with a hydrogen fluoride-pyridine reagent gave methyl fluoroshikimate directly, and this compound exhibits a low level of inhibition against one of the enzymes of the pathway.

It is suggested that this synthesis may be made enantiospecific by the use of enzymes, and some preliminary work is described.

Some unusual 1,4-addition reactions of thiophenol to synthetic intermediates are also reported as part of two unsatisfactory routes to the key epoxide.

The second part of the thesis describes approaches to 3- and 4- substituted analogues of shikimic acid from epoxide precursors. From empirical examples, it is indicated how the regio- and stereoselectivity of the products resulting from nucleophilic epoxide opening might be predicted and controlled.

The synthesis of 5-*epi*-shikimic acid by complementary routes is also demonstrated,

but under the conditions employed this compound could not be made in an enantiospecific form.

ABBREVIATIONS

Ac - acetyl

ADP - adenosine diphosphate

AIBN - azo-bis (isobutyronitrile)

atm - atmosphere

ATP - adenosine triphosphate

Bn - benzyl

B.p. - boiling point

t-Bu - *tert*-butyl

Bz - benzoyl

C.I. - chemical ionisation

COSY - correlation spectroscopy

m-CPBA - *meta*-chloroperbenzoic acid

DAHP - 3-deoxy -D-*arabino*-heptulosonate-7-phosphate

DBU - 1,8-diazabicyclo [5.4.0] undec-7-ene

d.e. - diastereomeric excess

DMAP - dimethyl aminopyridine

DME - dimethoxyethane

DMF - N,N-dimethylformamide

E1 - unimolecular elimination

e.e. - enantiomeric excess

E.I. - electron impact

EPSP - 5-enolpyruvyl-shikimate-3-phosphate

Et - ethyl

FAB - fast atom bombardment

g.l.c. - gas liquid chromatography

h - hour

HOMO - highest occupied molecular orbital

HMDS - 1,1,1,3,3,3-hexamethyldisilazane

HMPA - hexamethylphosphoramide

h.p.l.c. - high performance liquid chromatography

Hz - Hertz

i.r. - infra-red

J - coupling constant

LDA - lithium di-isopropylamide

LUMO - lowest unoccupied molecular orbital

Me - methyl

mg - milligram

min - minute

mmol - millimole

m.p. - melting point

Ms - mesyl (methane sulphonyl)

m.s. - mass spectrum

m/z - mass : charge ratio

NAD - nicotinamide adenine dinucleotide

NADPH - nicotinamide adenine dinucleotide phosphate, reduced

NBS - N-bromosuccinimide

n.m.r. - nuclear magnetic resonance

n.O.e. - nuclear Overhanser effect

NOEDS - n.O.e. difference spectroscopy

Nu - nucleophile

PEP - phosphoenol pyruvate

Ph - phenyl

PLE - pig liver esterase

p.p.m. - parts per million

R_F - retention factor

RT - room temperature

S_N2 - bimolecular nucleophilic substitution

S-3-P - shikimate-3-phosphate

TBDMS - *tert*-butyldimethylsilyl

Tf - triflyl (trifluoromethanesulphonyl)

THF - tetrahydrofuran

TMS - trimethylsilyl

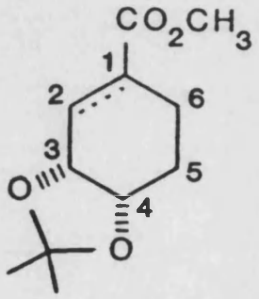
Tr - trityl (triphenylmethyl)

Ts - tosyl (*p*-toluenesulphonyl).

NOMENCLATURE

The nomenclature of cyclohexenes and related compounds referred to in this thesis is based, for the most part, on shikimic acid nomenclature, even though this may not necessarily conform to the IUPAC convention. This allows continuity and permits analysis of any compound without the need for reference to the nomenclature for that particular structure. Furthermore it enables direct comparisons to be made between compounds and facilitates the comparison of ^{1}H and ^{13}C n.m.r. data.

The numbering system employed labels the carboxylate substituted carbon as C-1, and proceeds anti clockwise around the ring (usually through the double bond) :



Bicyclic compounds are labelled in conformity with the usual IUPAC rules.

CONTENTS

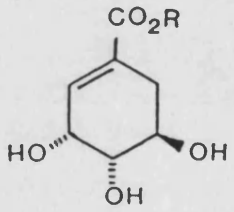
INTRODUCTION

CHAPTER 1 : INTRODUCTION

A. The Shikimic Acid Pathway[1-3]

1. Introduction

Aromatic compounds are ubiquitous in Nature and arise biosynthetically from two pathways : one is from the condensation of acetyl Co-A molecules *via* hypothetical polyketide intermediates[3,4]; the other is more significant in autotrophic organisms and is known as the shikimic acid pathway. The latter operates to yield the three aromatic amino acids phenylalanine, tyrosine and tryptophan, as well as *p*-amino- and *p*-hydroxybenzoic acids. These are important compounds in their own right, but also act as precursors for a host of secondary metabolites.



(1) R = H

(2) R = CH_3

Shikimic acid (1) was first isolated in 1885 from the Oriental plant *Illicium religiosum*, and it was from the Japanese name for this plant, shikimi-no-ki, that its name was coined. At that time, the significance of shikimic acid was not fully appreciated, and it was only through the elegant work of Davis[5] in the 1950's that its true importance was realised.

Davis obtained a series of mutants of *Escherichia coli* which would not grow without the addition of certain aromatic substances, including the aromatic amino acids. He found that the requirement for all these substances could be met by the

addition of a single compound - shikimic acid. Evidently these mutants had the biosynthetic pathway blocked at one or more of the early stages. Subsequent workers showed that the pathway has its origins in carbohydrate metabolism, and they also established the linear sequence of the intermediates involved. Thus the common pathway was formulated (Fig. 1-1).

2. The Common Pathway

The details of the early steps in the pathway have long been a source of debate, [6a] but the nature and sequence of intermediates are not disputed.[6]

Initially glucose is degraded into D-erythrose-4-phosphate (3) and phosphoenolpyruvate (PEP), and these then combine in an aldol-like condensation to afford 3-deoxy-D-*arabino*-heptulosonate-7-phosphate (DAHP) (4). The reaction is mediated by at least three isoenzymes, known broadly as DAHP synthetases, which are controlled by feedback inhibition from one of the three products - phenylalanine, tyrosine or tryptophan. Cyclisation of DAHP gives 3-dehydroquinate (5), which dehydrates to give 3-dehydroshikimate (6) under the influence of the enzyme 3-dehydroquinate dehydratase. Reduction of (6) to shikimate itself, followed by regioselective phosphorylation affords shikimate-3-phoshate (S-3-P) (7). Condensation of (7) with another molecule of PEP is catalysed EPSP synthetase, and yields 5-enolpyruvyl-shikimate-5-phosphate (EPSP) (8). The last step of the common pathway involves the 1,4-conjugate elimination of phosphoric acid from EPSP by chorismate synthetase. This affords chorismate (1), which is the pivotal point from which the pathway diverges.

Since the shikimic acid pathway is vital for the metabolism of plants and lower organisms, but does not operate in animals, it may allow the development of herbicides, and of selective fungicides or bacteriacides, which are non-toxic to animals.

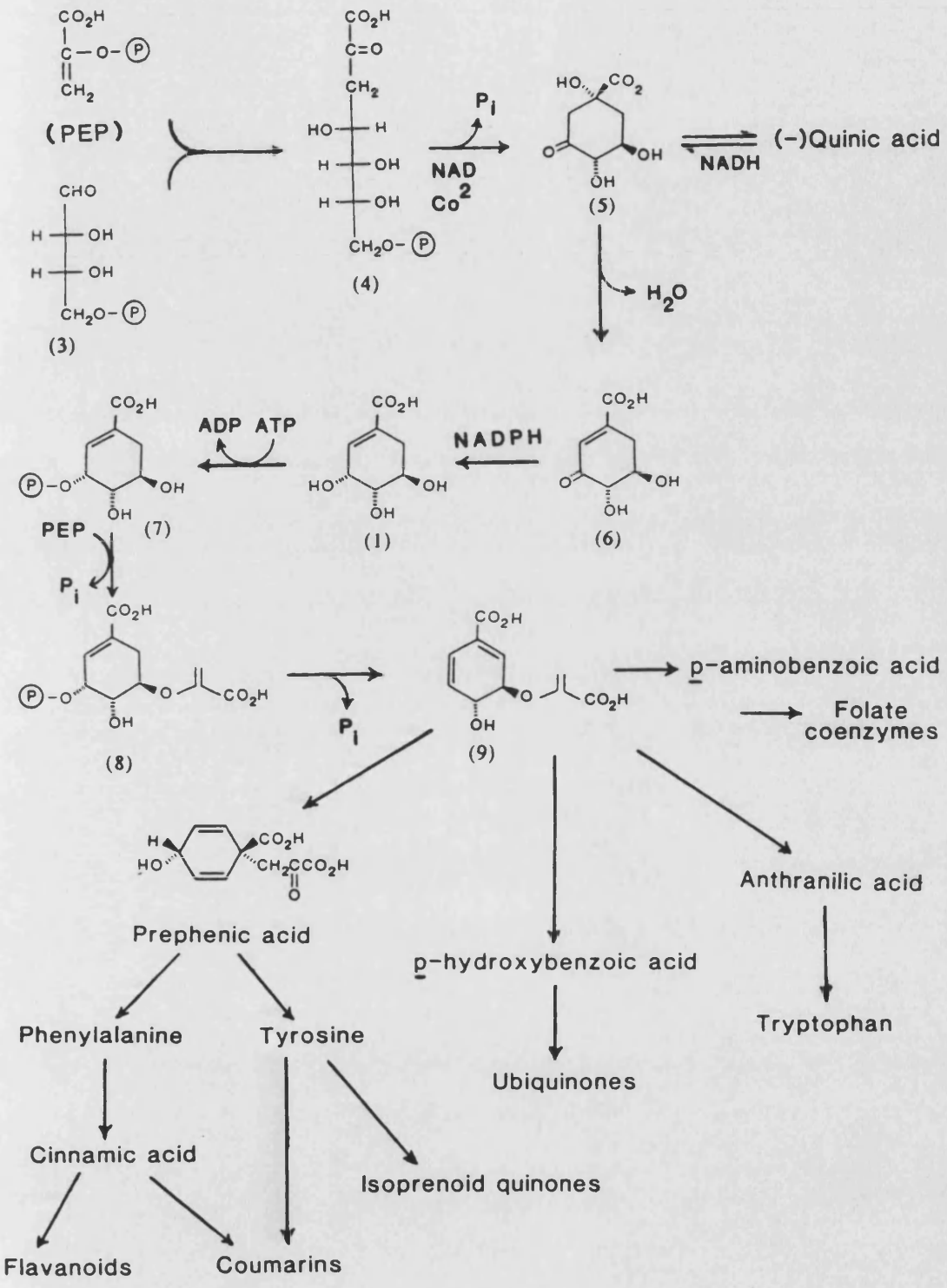


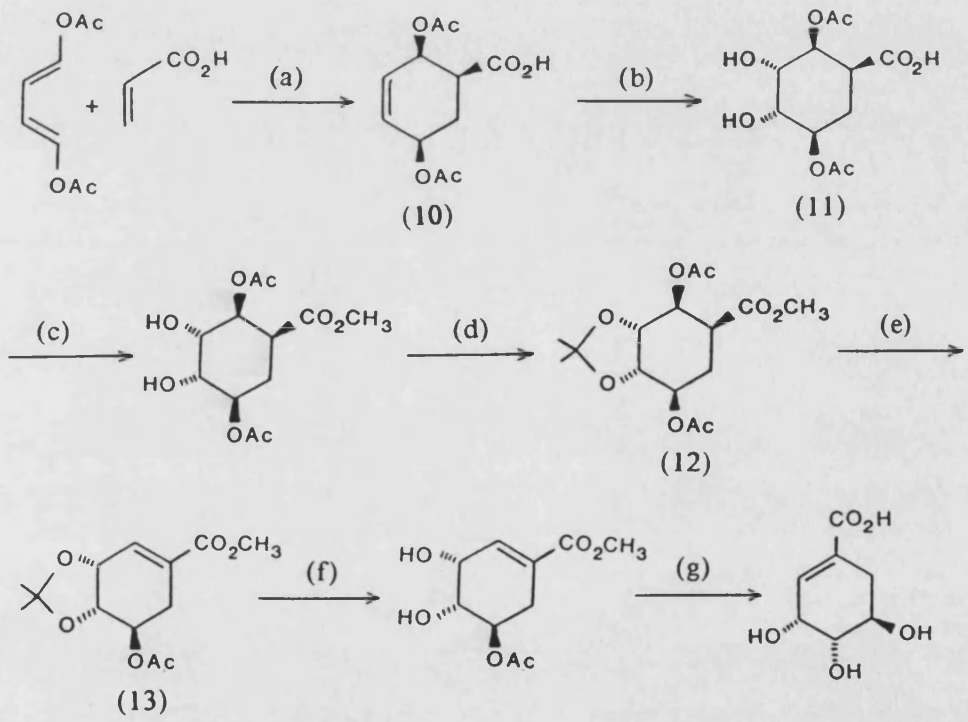
Fig. 1-1 The Shikimic Acid Pathway

B Racemic Total Syntheses

Most of the racemic syntheses of shikimic acid employ a Diels-Alder reaction to form the basic six carbon ring. In fact, of the nine groups who have published in this area, only two have employed alternative strategies.

1. Raphael *et al.*

The first successful synthesis of shikimic acid was achieved by Raphael *et al.*



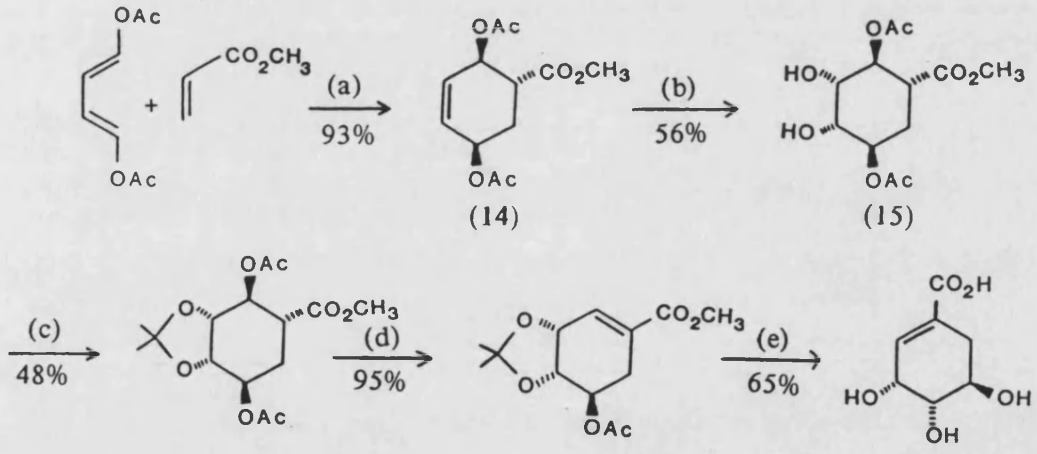
(a) 85-90°C, 30h; (b) OsO_4; (c) CH_2N_2; (d) Me_2CO, HCl; (e) MgO, 290°C; (f) H^+; (g) HO^- ; H^+

Fig. 1-2

using the Diels-Alder adduct of 1,4-diacetoxy-1,3-butadiene and acrylic acid as the starting material[7] (Fig. 1-2). The relative stereochemistry of the adduct (**10**) was assumed to be *cis* as shown and so dihydroxylation from the least hindered face set up the correct relative stereochemistry at the 3-, 4- and 5- positions in (**11**). After protection of both the hydroxyl and carboxylate moieties pyrolysis of (**12**) over magnesium oxide afforded the α,β-unsaturated ester (**13**), which was deprotected in two steps to give (±)-shikimic acid. Resolution was subsequently effected *via* the quinine methohydroxy salts of the shikimic acid triacetates.

2. Smissman *et al.*

An essentially identical route was reported simultaneously by Smissman *et al.*[8] (Fig. 1-3). These workers used a Diels-Alder reaction between 1,4-diacetoxy-1,3-



(a) xylene, reflux; (b) OsO_4; (c) Me_2CO, HCl; (d) 285°C, $7x10^{-3}$mmHg;
(e) H^+; HO^-; H^+

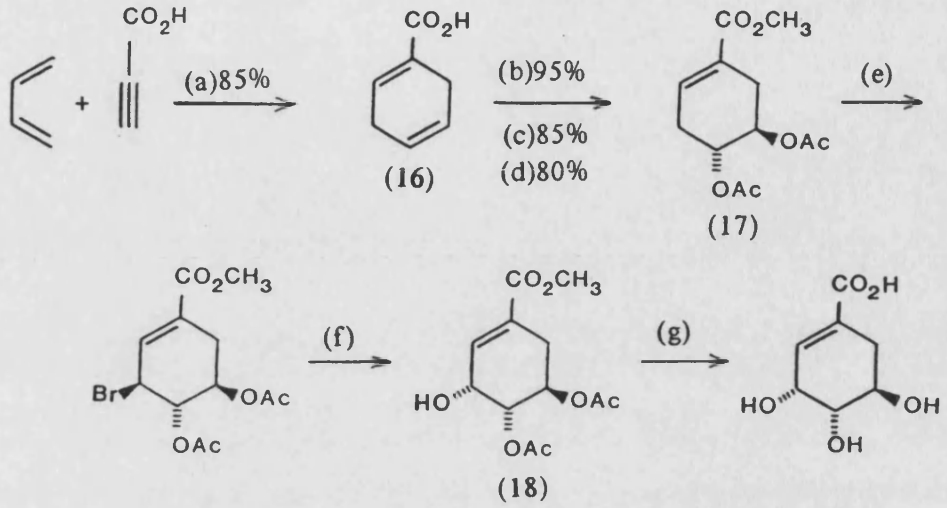
Fig. 1-3

butadiene and methyl acrylate to give the adduct (14). They assigned the relative stereochemistry of the methoxycarbonyl as α[9], in contradiction to that proposed by Raphael. *Cis*-hydroxylation afforded a diol (15) which, after protection, could not be converted into a shikimic acid derivative by base catalysed elimination of acetic acid. The elimination did occur on pyrolysis, and these results were incorrectly taken as evidence for a *syn* relationship between the acetoxy and adjacent hydrogen on C-1. The synthesis was completed in much the same way as Raphael's, and resolution effected using α-phenylethylamine.

Raphael was able to effect base mediated elimination (albeit in low yield) and justified his original stereo assignments of the adduct (10) by double resonance ^{1}H n.m.r. experiments[10] on the derivative (12), and his conclusions were supported by group of workers at Harvard[11]. Smissman was consequently obliged to amend his original assignment[12].

3. Grewe and Hinrichs

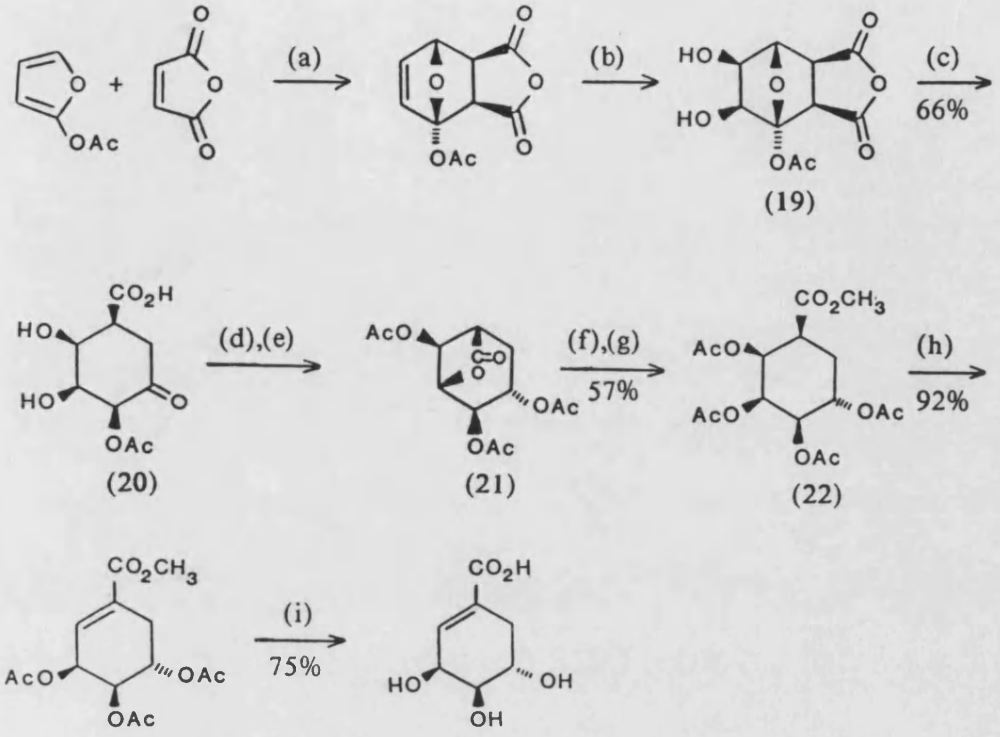
Grewe and Hinrichs also reported a synthesis[13] which employed a Diels-Alder reaction to assemble the carbocyclic ring (Fig. 1-4). Here the adduct (16) of butadiene and propynoic acid was methylated, epoxidised and hydrolysed to give a *trans* diol. The product was acetylated to give (17), and an allylic bromination step used to introduce a bromine atom at C-3, *trans* to the C-4 acetoxy group. Consequently inversion of configuration at this site by displacement of bromide gave a product with the correct relative stereochemistry (18). This left only the need for simple deprotection steps to obtain the racemic shikimic acid. The overall yield from adduct (16) was 20%.



(a) 130-140°C, 9h; (b) MeOH, H_2SO_4; (c) HCO_3H; hydrolysis; (d) Ac_2O, pyridine; (e) NBS, CCl_4; (f) AgOAc, H_2O; (g) KOH
Fig. 1-4

4. Doshi

Another synthesis was accomplished by Doshi[14] (Fig. 1-5) and was based on an earlier, unsuccessful attempt outlined by Raphael *et al.*[7] In this work the Diels-Alder adduct of 2-acetoxyfuran and maleic anhydride was hydroxylated, and the product *exo-cis*-diol (**19**) stirred in water for three days. This treatment resulted in opening of the hemiketal acetate ring system, acetyl migration and mono decarboxylation. The ketone (**20**) formed from this sequence of reactions was reduced, and under acetylation conditions gave the lactone (**21**). The lactone was opened with methanol-hydrochloric acid, and acetylation of the free hydroxyl gave a fully protected compound (**22**), which on pyrolysis afforded shikimic acid triacetate. Deprotection under basic conditions yielded (±)-shikimic acid.

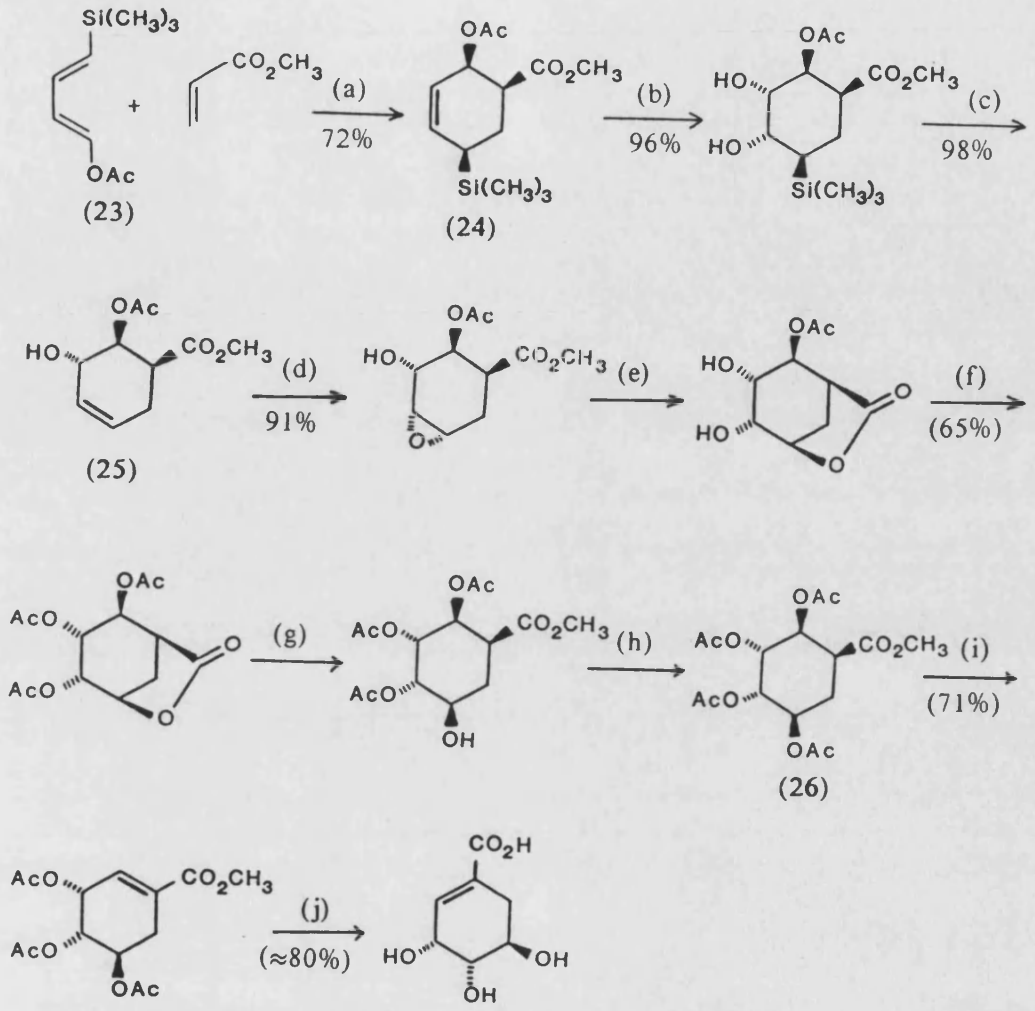


(a) xylene, reflux; (b) OsO_4, H_2O_2; (c) H_2O, 3 days; (d) $NaBH_4$; (e) Ac_2O; (f) HCl, MeOH; (g) Ac_2O; (h) 256-8° C, 0.25mmHg, 20 mins.; (i) HO^-

Fig. 1-5

5. Koreeda *et al.*

The next total synthesis[15] was not reported until 1982 when Koreeda prepared shikimic acid in order to illustrate the use of a novel diene (23) in natural product synthesis (Fig. 1-6). Upon reaction with methyl acrylate this reagent gave the cycloadduct (24), which was hydroxylated and converted into the alkene (25). Treatment of (25) with *m*-chloroperoxybenzoic acid (*m*-CPBA) gave an α-epoxide stereoselectively. After lactonisation, which set up the 4,5 *trans* diol system



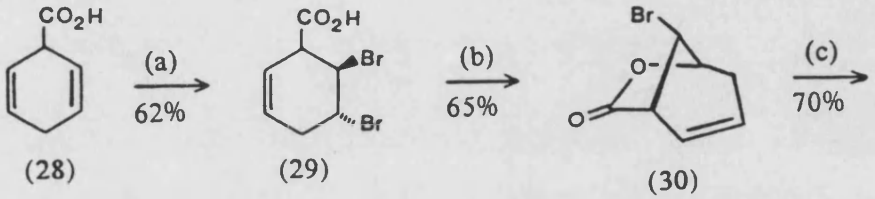
a) xylene, reflux; (b) OsO_4,N-methylmorpholine-N-oxide; (c) *p*-TSA, C_6H_6, reflux; (d) *m*-CPBA; (e) LiOH; (f) Ac_2O, pyridine; (g) MeOH, HCl; (h) Ac_2O, pyridine; (i) DBU; (j) HO^-

Fig. 1-6

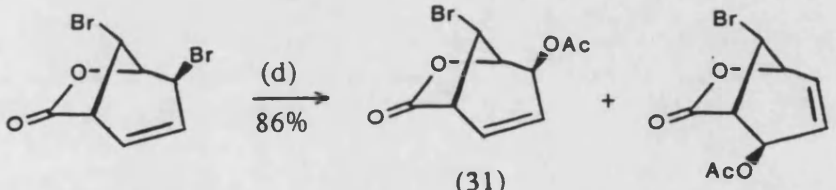
necessary for shikimic acid, the remaining steps to the target molecule were straightforward, with the overall yield from (23) being 23%. It is interesting to note that the tetracetate (26) allows the correct antiperiplanar conformation for base mediated elimination of acetic acid. It seems likely that the isopropylidine group in Raphael's compound (12) disfavours attainment of the required transition state geometry.

6. Ganem *et al.*

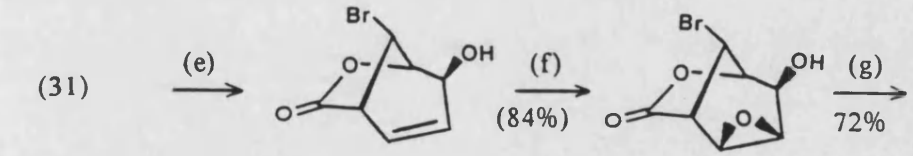
In the same year Ganem published a synthesis of (±)-(1)[16] which is noteworthy in that it was the first strategy not to employ a Diels-Alder reaction (Fig. 1-7). In this work the cyclohexadiene (28) was brominated, and the major product of this reaction (29) cyclised to afford the bromolactone (30). An allylic bromination of this, and treatment with sodium acetate gave a mixture of two bromo acetates (**31** and **32**). Acid hydrolysis of (**31**) gave an allylic alcohol which was stereoselectively epoxidised to furnish a β-epoxide. Radical debromination of this epoxide yielded the lactone (33), and treatment of (33) with potassium hydroxide afforded racemic shikimic acid in an overall yield of 13%.

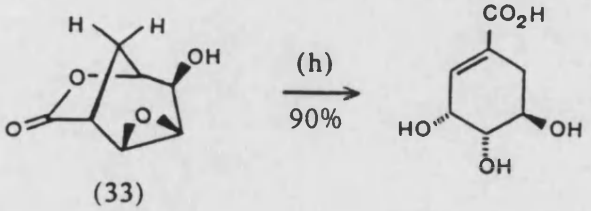


(28) (29) (30)



(31) (32)





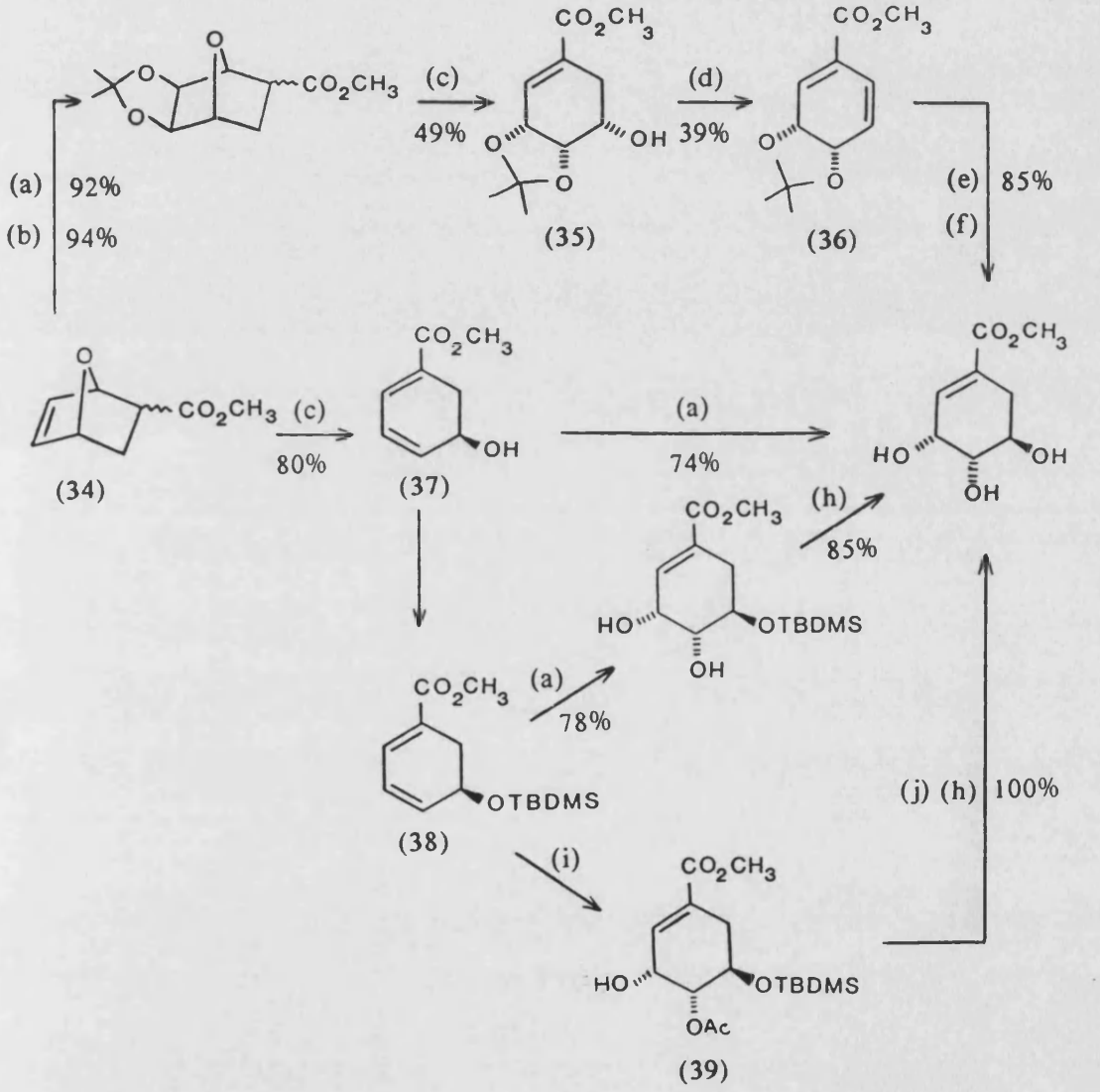
(33) 13% overall yield

(a) Br_2, CH_2Cl_2; (b) $NaHCO_3$; (c) NBS,CCl_4 , $(PhCO_2)_2$, reflux; (d) 4 equiv. NaOAc, HMPA; (e) Hydrolysis; (f) CF_3CO_3H, $ClCH_2CH_2Cl$, reflux; (g) Bu_3SnH, AIBN; (h) 1.25 equiv KOH, 4:1 MeOH-H_2O.

Fig. 1-7

7. Campbell, Sainsbury *et al.*

Recently Campbell and Sainsbury have reported a number of brief syntheses of methyl shikimate[17-19] (Fig. 1-8), all starting with the Diels-Alder adduct (**34**) of furan and methyl acrylate. One route involves *cis*-hydroxylation of (**34**), protection as



(a) OsO_4, H_2O_2; (b) $Me_2C(OMe)_2$, *p*-TSA; (c) $LiN[SiMe_3]_2$, -78°C; (d) DEAD, PPh_3; (e) B_2H_6.THF, H_2O_2; (f) Ion exchange resin (H^+); (g) TBDMSOTf, 2,6-lutidine; (h) Bu_4NF; (i) AgOAc, I_2, aq. AcOH, 70°C; (j) aq. NH_3, MeOH.

Fig. 1-8

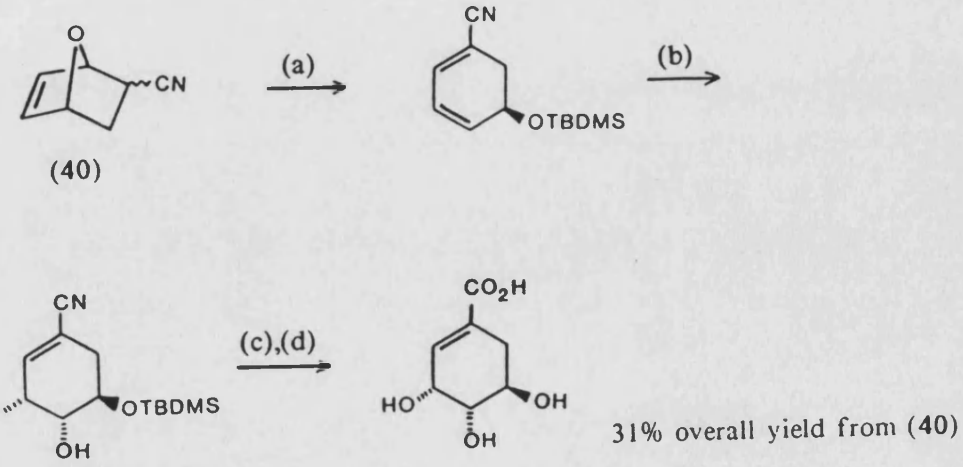
the acetonide, and base mediated opening of the oxabicyclic ring. The product of this sequence of reactions is the acetonide of methyl 5-*epi*-shikimate (35), but unfortunately direct attempts to invert the stereochemistry of the 5-hydroxyl were unsuccessful. It is interesting to note, however, that under Mitsunobu conditions dehydration occurred to yield the diene (36), which, on hydroboration, oxidation and deprotection afforded (±)-methyl shikimate.

A complementary route reversed the order of reactions. Thus initial opening of the adduct (34) with lithium hexamethyldisilazide gave the diene (37), whereupon hydroxylation gave methyl shikimate (2), and methyl 5-*epi*-shikimate in a ratio of 5:1 (overall yield of (2) being 23%). The selectivity of the hydroxylation reaction was increased by first protecting the hydroxyl as a bulky *t*-butyldimethylsilyl ether (38), which, after osmylation and deprotection gave (2) in 26% yield from (34).

Another variation employed a "wet" Prévost reaction upon (38). In this case hydrolysis of the product acetate (39) and the subsequent deprotection steps were virtually quantitative, giving rise to a similar overall yield of 26%, but avoiding the use of the toxic and expensive osmium tetroxide.

8. Rodrigo *et al.*

A communication in the same year by Rodrigo *et al.*[20] described an essentially similar plan. In addition to duplicating the Campbell-Sainsbury work from the adduct



(40)

31% overall yield from (40)

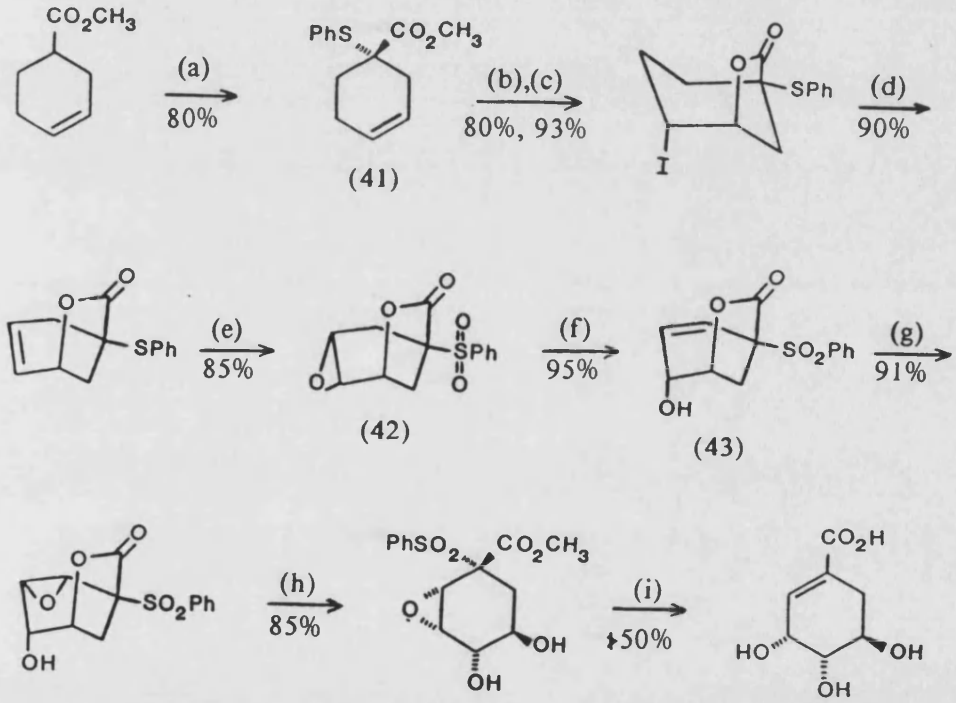
(a) LDA; (b) OsO_4, pyridine; (c) Bu_4NF; (d) HO^-,H_2O

Fig. 1-9

(34), these workers completed a synthesis of (±)-shikimic acid from the furan-acrylonitrile adduct (40) using similar techniques (Fig. 1-9). The overall yield in this case was 31%.

9. Bartlett *et al.*

The most recent racemic synthesis to date is that of Bartlett[21], and is only the second which does not employ a Diels-Alder approach (Fig. 1-10(a)). Starting with methyl 3-cyclohexenecarboxylate these workers obtained the α-phenylthioether (41) by



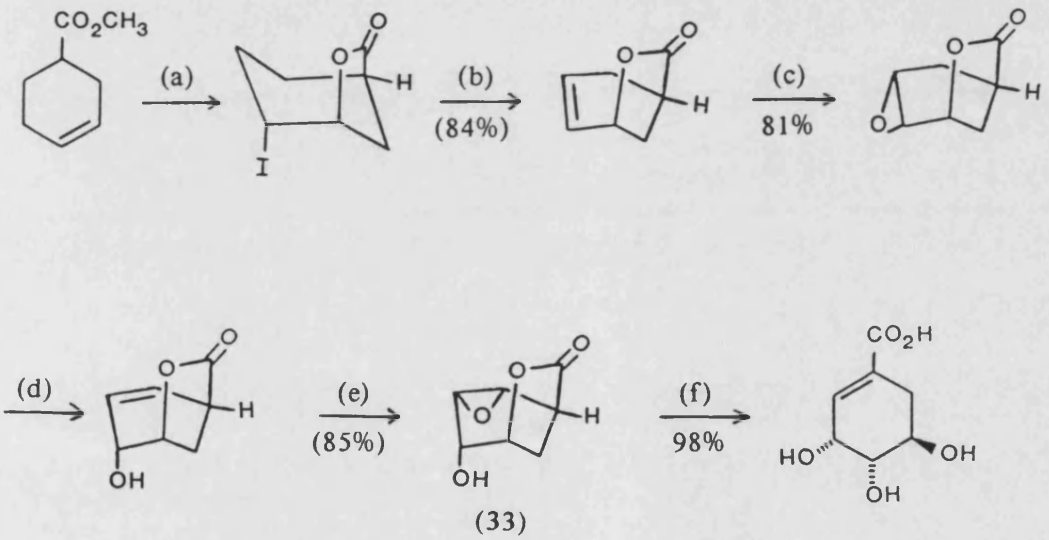
(a) PhSSPh; (b) NaOH; HCl; (c) KI_3; (d) DBU; (e) F_3CCO_3H;

(f) PPh_3(cat.), TMSBr; DBU; (g) F_3CCO_3H; (h) K_2CO_3, MeOH;

(i) Al(Hg), wet THF

Fig. 1-10(a)

reaction with diphenyl disulphide, with the intention of introducing the 3-hydroxyl moiety of shikimic acid via a Mislow-Evans rearrangement. Iodolactonisation of (41) followed by dehydroiodination and peracid oxidation afforded the epoxysulphone (42). The unwanted oxidation of sulphur at this point forced Bartlett to abandon his



(a) $NaHCO_3$, I_2,KI, H_2O; (b) DBU, reflux; (c) 3,5-dinitroperbenzoic acid;

(d) PPh_3, TMSBr; DBU; (e) 3,5-dinitroperbenzoic acid, reflux; (f) MeOH, K_2CO_3

Fig. 1-10(b)

original route. However, the synthesis was continued in a modified approach by isomerisation of (42) to the allylic alcohol (43), in a one-pot reaction. Stereoselective

epoxidation, lactone fission, and a relatively inefficient reductive elimination step completed the synthesis.

Without the need for sulphur in the synthetic intermediates, a superior route was completed on the unsubstituted series of compounds (Fig. 1-10(b)) utilising the same sequence of reactions. Having dispensed with the sulphur functionality, the reductive elimination step - a weak link in the previous route - was now unnecessary, and racemic methyl shikimate was obtained in >50% overall yield.

The lactone (33) is identical to that reported by Ganem *et al.* (see Fig. 1-7), who obtained shikimic acid directly by treatment of (33) with sodium hydroxide in methanol.

C Total and Partial Enantiospecific Syntheses

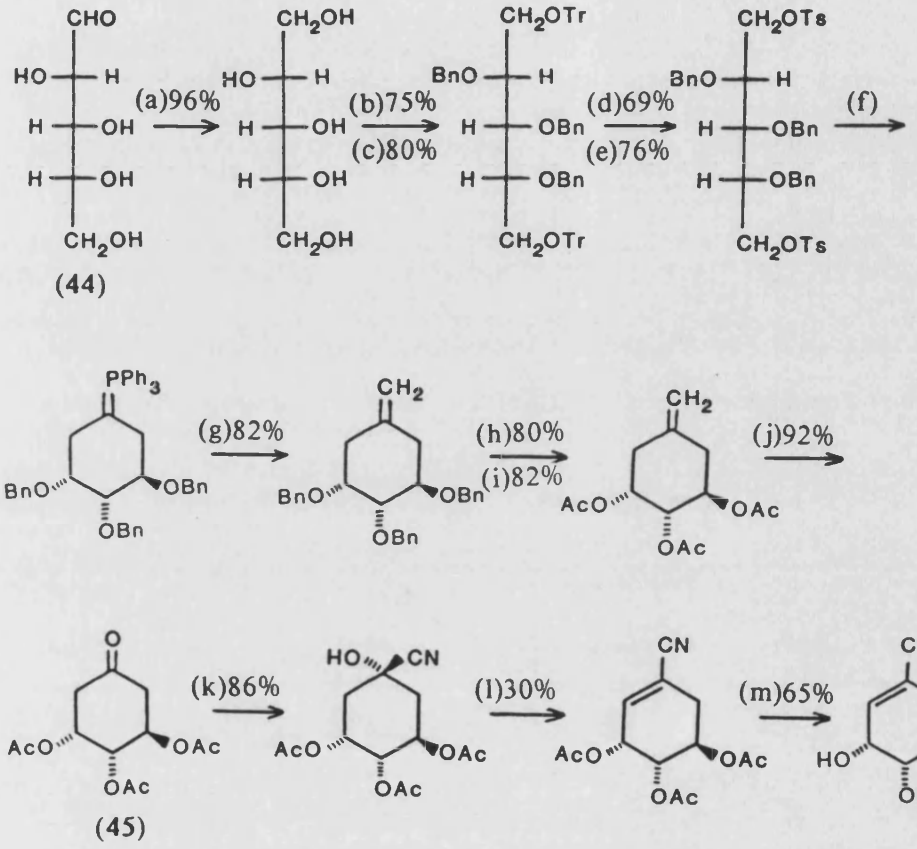
1. Syntheses from Sugars

In the field of natural product synthesis it has become fashionable to use carbohydrates as readily available sources of chiral starting material. Shikimic acid is no exception, and a number of such syntheses have been completed. These are outlined below.

(i) D-Arabinose

The first total enantiospecific synthesis of shikimic acid was that of Bestmann and Heid[22] (Fig. 1-11). A lengthy series of manipulations, beginning with D-arabinose (44), eventually lead to a single enantiomer of a triacetoxycyclohexanone (45), a key intermediate in an earlier synthesis of shikimic acid from quinic acid by Grewe[23] (see Fig. 1-21). Following Grewe's original procedure, the ketone (45) was converted into a cyanohydrin, and this was dehydrated and hydrolysed to give (-)-(1).

Unfortunately the utility of the Bestmann-Heid route is limited by the number of steps involved, and this is reflected in the low overall yield (2%).



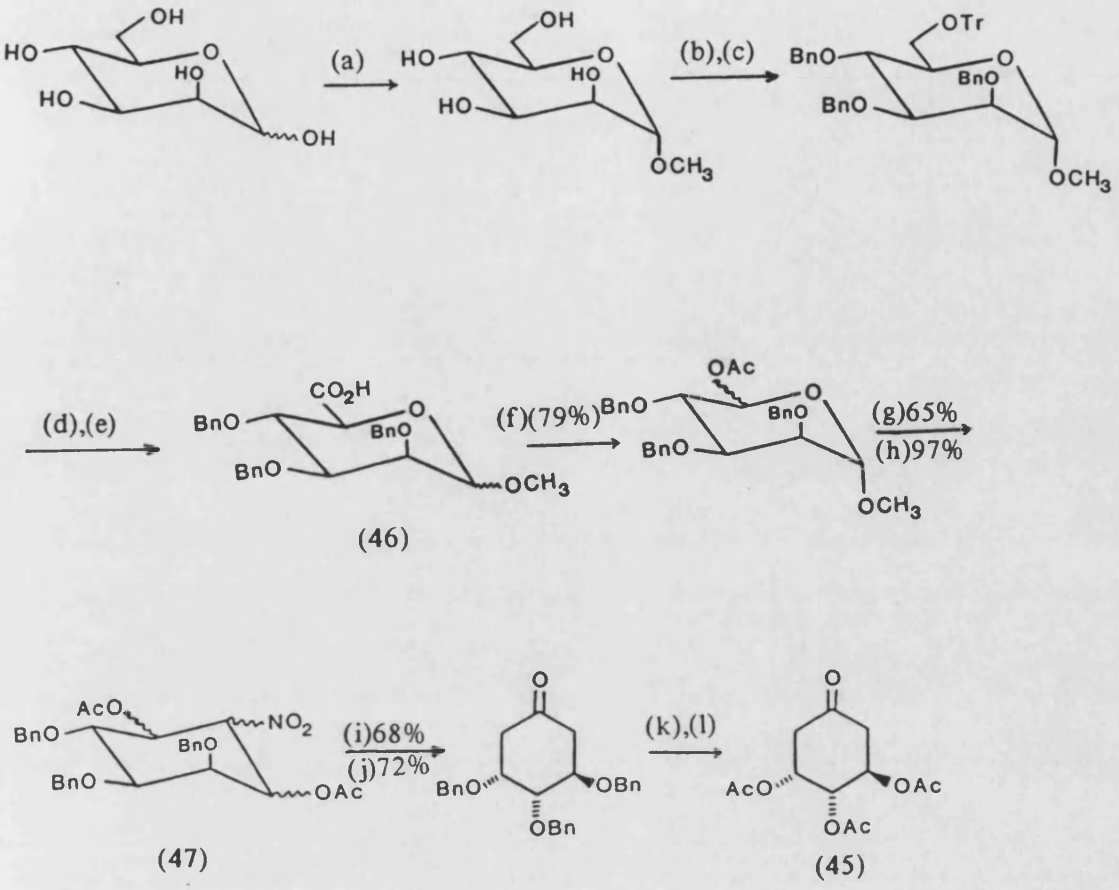
Overall yield ≈2%

(a) H_2/Ni; (b) TrCl, pyridine; (c) BnCl, KOH; (d) 70% AcOH; (e) TsCl, pyridine; (f) 3 equiv. CH_2=PPh_3; (g) H_2CO; (h) Na/NH_3; (i) Ac_2O, pyridine; (j) OsO_4, $NaIO_4$; (k) HCN; (l) $POCl_3$, pyridine; (m) NaOH, H_2O.

Fig. 1-11

(ii) D-Mannose

Grewe's triacetoxycyclohexanone (45) was also the target for Kitagawa *et al.*[24], and indeed they did not take their synthesis further than this compound (Fig. 1-12). They chose to use D-mannose to obtain the correct chirality at C-3, C-4 and C-5,

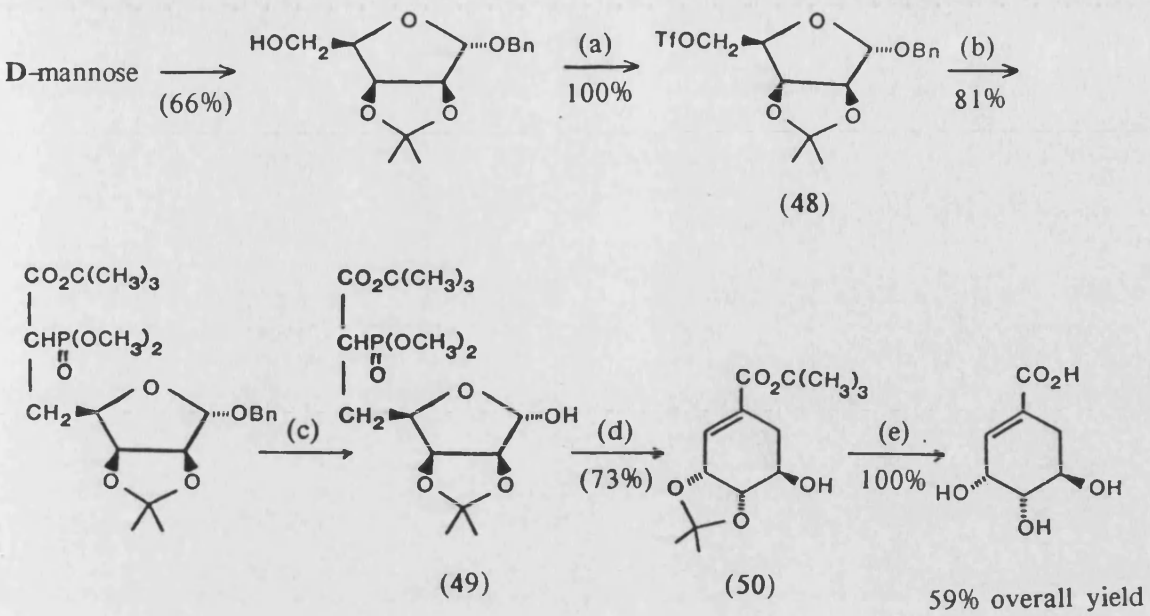


(a) MeOH, AcCl; (b) TrCl, pyridine; (c) BnCl, NaH, DMF; (d) 5% aq. H_2SO_4; (f) $Pb(OAc)_4$; (g) $MeNO_2$, NaOMe/MeOH; (h) Ac_2O, BF_3. Et_2O; (i) $NaBH_4$; (j) $TiCl_3$, $AcONH_4$; (k) H_2,10% Pd-C; (l) Ac_2O,MeOH.

Fig. 1-12

converting this to a suitably functionalised and protected intermediate (46), which was oxidatively decarboxylated and treated with nitromethane. After acetylation the resulting nitrocyclitols (47) were treated with sodium borohydride to effect a reductive elimination of both acetoxy groups. A series of straightforward deprotection and protection steps were all that remained in order to reach the target molecule (45).

D-mannose was also the starting material for one of the most notable syntheses of (-)-shikimic acid - the elegant approach of Fleet *et al.*[25] (Fig. 1-13). Here the 5-*O*-triflyl derivative of protected D-mannose (48) was treated with the sodium salt of *t*-butyl dimethoxyphosphorylacetate, and the products, on hydrogenolysis and treatment with base, gave the acetonide of *t*-butyl shikimate (50). The key step here is an



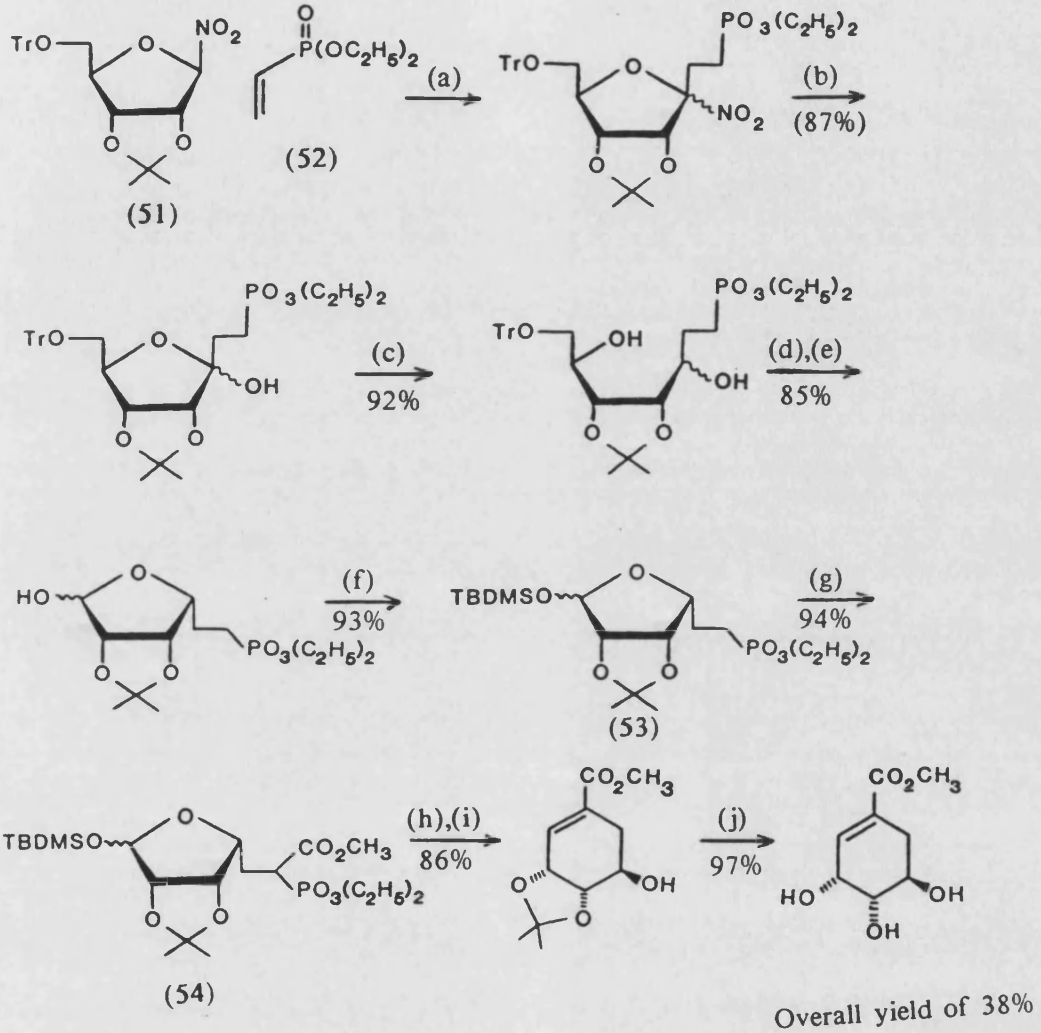
(a) $(F_3CSO_2)_2O$; (b) $[(MeO)_2P(O)CHCO_2t\text{-Bu}]$ Na, DMF, 15-crown-5; (c) H_2, Pd-C; (d) NaH; (e) 60% F_3CCO_2H

Fig. 1-13

intramolecular Wadsworth-Emmons olefination of the hemiacetal (49). After deprotection, (-)-(1) was obtained in an overall yield of 59% from D-mannose, illustrating the expediency of this approach.

(iii) D-Ribose

A similar base catalysed intramolecular cyclisation formed the basis of a related strategy (Fig. 1-14) by Vasella[26]. The synthesis began with Michael addition of the anion of nitroribose derivative (51) to the vinyl phosphonate (52), and the product manipulated to afford a protected hemiacetal (53). Treatment of (53) with butyl-lithium and methyl chloroformate gave the esters (54), which, after desilylation, released the corresponding hemiacetal. This was not isolated, but was cyclised to give the acetonide of (-)-methyl shikimate upon treatment with base, and the latter converted smoothly to (-)-(2) with acid. Although the overall yield was 38% a number of non-stereoselective steps were involved, and several separations were necessary, making this route somewhat cumbersome.



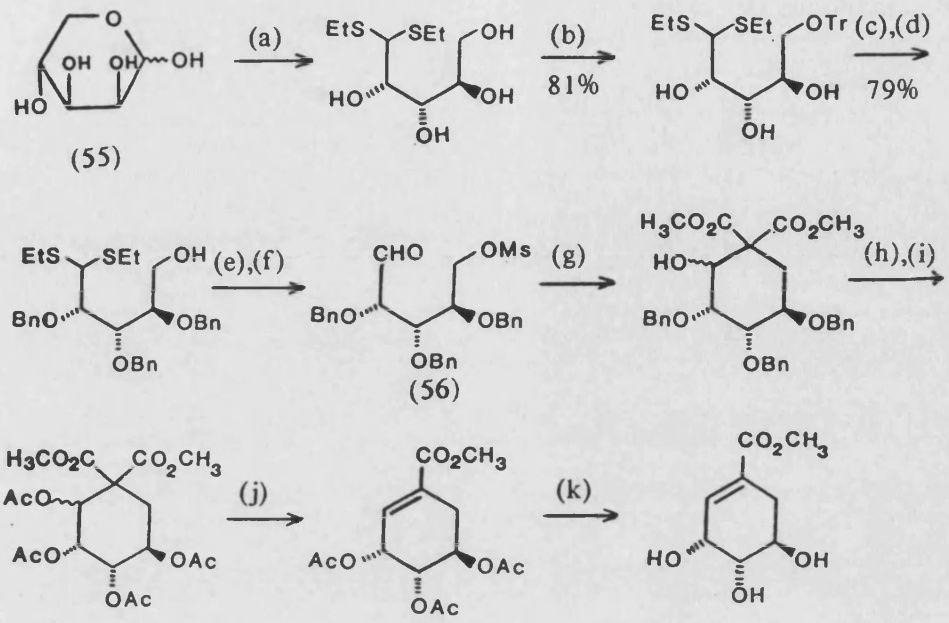
Overall yield of 38% from D-ribose

(a) $Bu_4NF.3H_2O$; (b) $HCONH_2$, 60°C, 24h; (c) $NaBH_4$; (d) $ZnBr_2$; (e) $NaIO_4$; (f) TBDMSCl; (g) BuLi; $ClCO_2Me$; (h) $Bu_4NF.3H_2O$; (i) NaOMe/MeOH; (j) H^+

Fig. 1-14

(iv) D-Lyxose

A novel approach to the carbocyclic ring system of shikimic acid from a carbohydrate was demonstrated by Suami and co-workers[27]. In this case the substrate sugar was D-lyxose (55), and the introduction of the two extra carbon atoms required



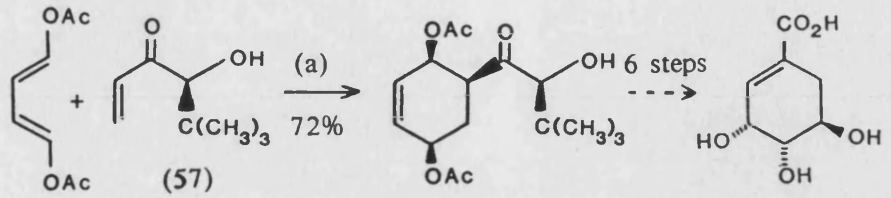
(a) EtSH, HCl; (b) TrCl; (c) BnBr, NaH; (d) *p*-TSA; (e) MsCl; (f) $HgCl_2$, $CaCO_3$; (g) $CH_2(CO_2Me)_2$, NaH; (h) 20% $Pd(OH)_2/C$, MeOH, reflux, 2 days; (i) Ac_2O; (j) aq. DMSO, NaCl, 125°C; (k) NaOMe/MeOH.

Fig. 1-15

came from reaction of the anion of dimethyl malonate with the protected pentose (56) (Fig. 1-15). Thus the problems of chain elongation and cyclisation were solved in a single step. The synthesis was carried through to (-)-methyl shikimate by acetylation, simultaneous thermal decarbomethoxylation and β-elimination of the acetoxy group, and deprotection. Unfortunately this route, like many preceding it, suffers from extensive protection and deprotection steps. These serve to extend the length, and with at least two particularly inefficient steps the overall yield is reduced.

2. Diels-Alder Approach

As part of a programme of asymmetric syntheses[28] Masamune chose to illustrate applications of chiral dienophiles with some examples of asymmetric Diels-Alder



(58)

>98% d.e.

(a) 0.5 equiv. $BF_3.Et_2O$

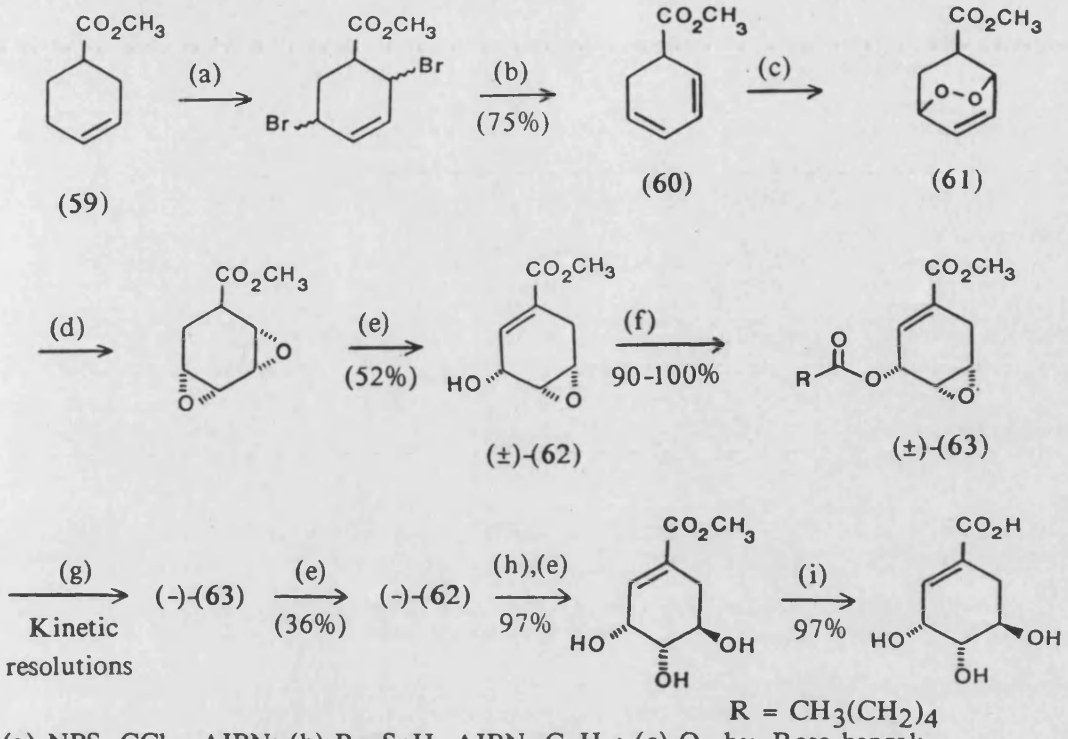
Fig. 1-16

reactions[29]. In one of these he completed the synthesis of (-)-shikimic acid (Fig. 1-16). Reacting 1,4-diacetoxy-1-3-butadiene with the chiral dienophile (57) he obtained the cycloadduct (58) in >98% d.e. as the only product in 72% yield. From then on the remainder of the synthesis followed the Raphael-Smissman route, but few details were given and it is difficult to make an objective assessment of this achievement in terms of a viable chiral synthesis of (1).

3. Enzyme Mediated Enantioselective Hydrolysis

A recent synthesis of (-)-shikimic acid is that of Berchtold *et al.*[30], which uses as its starting material the racemic epoxy alcohol (62) (Fig. 1-17). The preparation of (±)-(62), which had been outlined in a previous paper[31], involves several steps: double allylic bromination of methyl cyclohex-3-enecarboxylate (59) followed by radical induced dehalogenation and photo-oxidation of the product diene (60), yield the endoperoxide (61). Rearrangement of (61) gave a *bis*-epoxide which was isomerised to (62) in the presence of base.

Esterification of (±)-(62) with hexanoyl chloride afforded the esters (±)-(63). Kinetic resolutions of (±)-(63) was achieved with a variety of enzymes - the notable exception being pig liver esterase which preferentially removed the methyl ester with no observable kinetic resolution - leaving (-)-(63) unhydrolysed. Removal of the side chain ester by treatment with sodium methoxide in methanol yielded (-)-(62) in 36%



(a) NBS, CCl_4, AIBN; (b) Bu_3SnH, AIBN, C_6H_6; (c) O_2,hν, Rose bengal;
(d) $(Ph_3P)_2RuCl_2$, CH_2Cl_2; (e) NaOMe/MeOH; H^+;
(f) $CH_3(CH_2)_4COCl$, Et_3N, DMAP; (g) cholesterol esterase, H_2O,pH7.8, 0-5°C;
(h) 80%aq. AcOH; (i) NaOH; H^+.

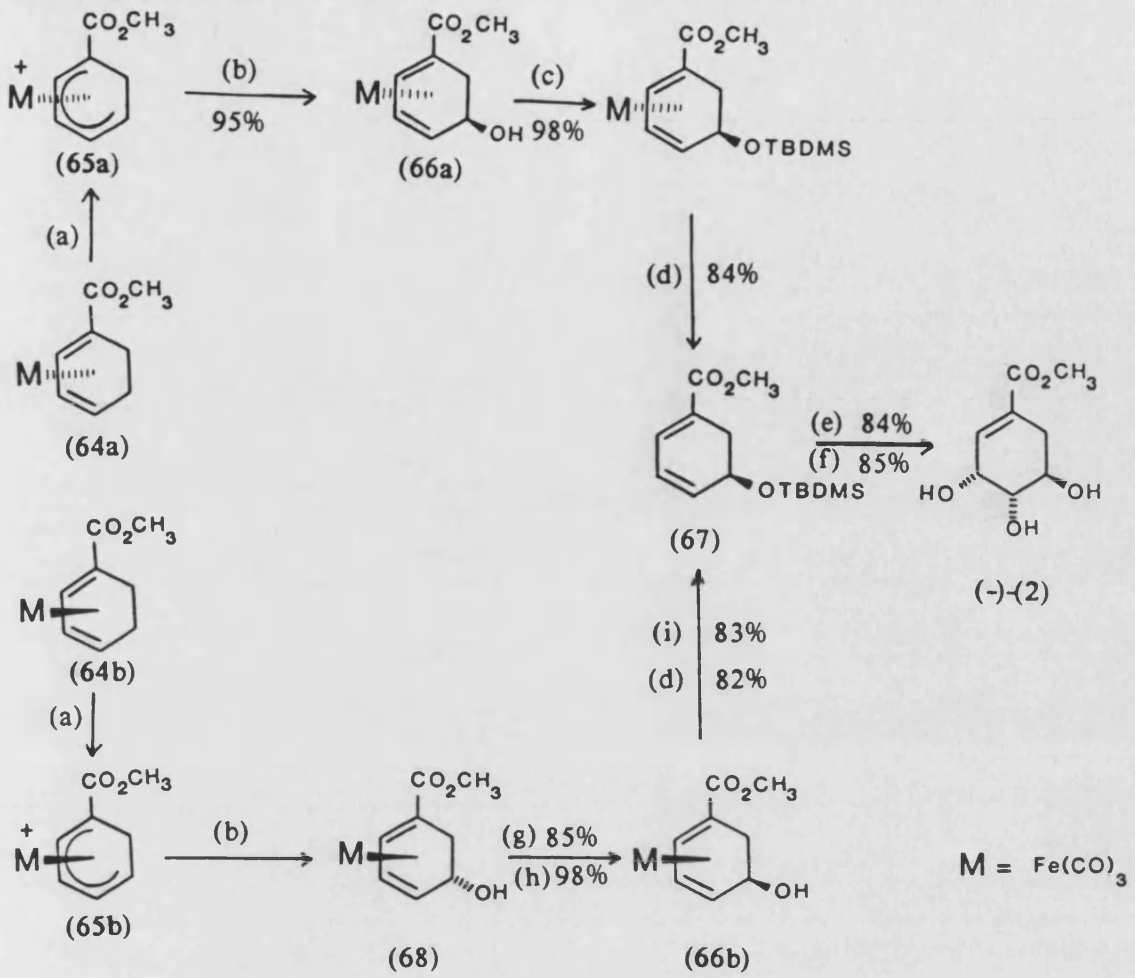
Fig. 1-17

yield (from a theoretical maximum of 50%) and 97% e.e. Conversion of (-)-(62) into (-)-(1) was then carried out by a known series[32] of simple, high yielding chemical transformations.

4. An Organometallic Approach

The most recent synthesis to date is that of Birch *et al.*[33] who prepared (-)-methyl shikimate from a chiral form of the Bath intermediate (37) (Fig. 1-18).

The presence of $Fe(CO)_3$ as a lateral control group allows either of the resolved enantiomeric complexes[34] (64a) and (64b) to be used in the synthesis of (-)- or (+)-shikimic acid. The former was synthesised directly from (64a) by first forming the cationic salt (65a) which reacts with nucleophiles solely at the 5-*exo*-position[35]. Thus reaction of (65a) with aqueous sodium hydrogen carbonate yielded the alcohol complex (66a).



(a) $Ph_3C^+PF_6^-$, CH_2Cl_2; (b) $NaHCO_3$, aq MeCN; (c) TBDMSCl, DMF, $(i\text{-Pr})_2NEt$; (d) Me_3NO,C_6H_6; (e) OsO_4, Me_2CO; (f) Bu_4NF, THF; (g) CrO_3, pyridine, CH_2Cl_2; (h) $NaBH_4$, $ZnCl_2$, Et_2O; (i) TBDMSOTf, DMF, $(i\text{-Pr})_2NEt$.

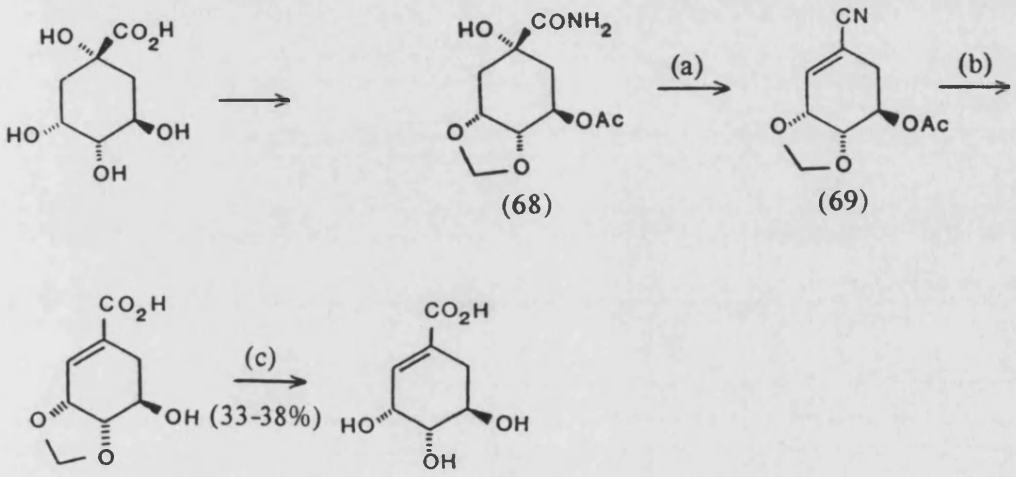
Fig. 1-18

Protection of the hydroxyl as a *t*-butyldimethylsilyl ether, and decomplexation gave the diene (67). This is a chiral form of the key intermediate reported by Campbell and Sainsbury *et al.*[17], and by using a slight modification of their procedure Birch was able to obtain (-)-(2). Alternatively, the enantiomeric complex (65b) was reacted with hydroxide ion to give the alcohol (68) which has the wrong absolute configuration at the 5-OH position. However, inversion of this by Jones oxidation, followed by stereospecific reduction using sodium borohydride and zinc chloride provided the desired isomer (66a), and the conversion to (-)-(2) was completed as before.

D. Syntheses From Quinic Acid

1. The Synthesis of Dangschat and Fischer

Although the resemblance between quinic acid and shikimic acid was noted very early on, unequivocal proof did not arise until the work of Dangschat and Fischer in 1950[36]. These workers accomplished a relatively simple conversion of (-)-quinic acid into (-)-shikimic acid* (Fig. 1-19).



(a) TsCl,pyridine,37°C, 7 days; (b) NaOH; H^+; (c) H_2SO_4

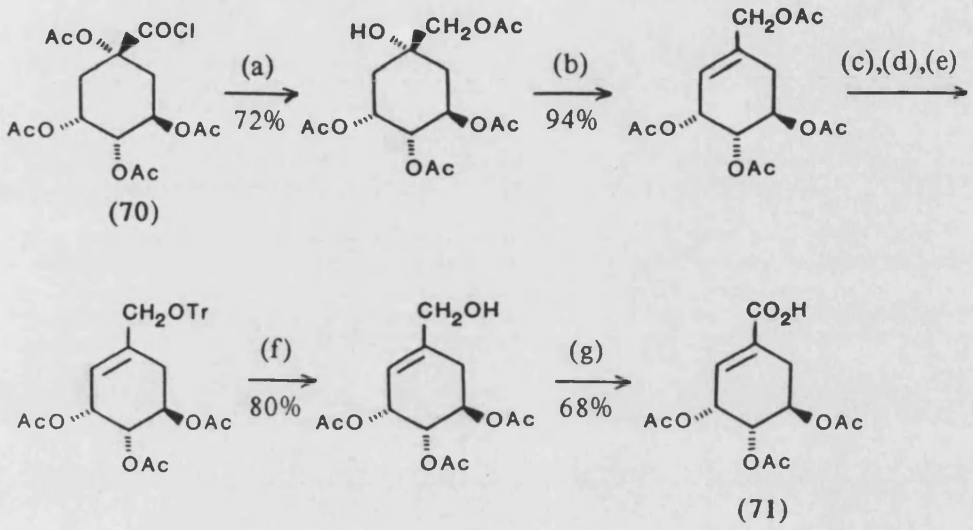
Fig. 1-19

A protected form of quinamide (**68**) was converted into the cyclohexene nitrile (**69**), which, upon hydrolysis and deprotection yielded (-)-shikimic acid.

* The reverse transformation has also been accomplished[37].

2. Partial Syntheses of Grewe *et al.*

Following this work Grewe *et al.* published two partial syntheses of (1) from quinate derivatives.

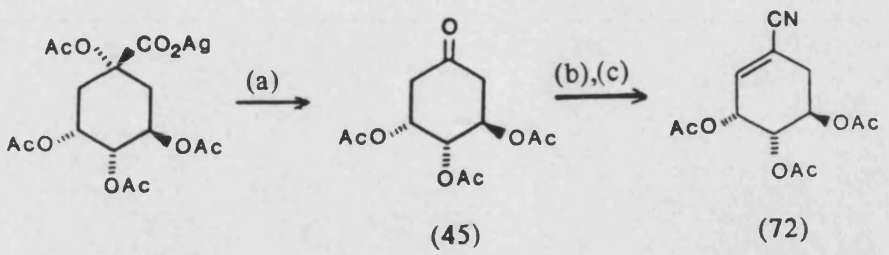


(a) $NaBH(OMe)_3$; (b) $POCl_3$, pyridine; (c) NaOMe, MeOH; (d) TrCl, pyridine; (e) Ac_2O; (f) H^+; (g) CrO_3.

Fig. 1-20

The first[38] (Fig. 1-20) is quite conventional, and uses the acid chloride of tetra-acetyl-quinate (70) as its starting material. It does, however, involve an interesting first step whereby treatment of (70) with sodium trimethoxyborohydride effects a reduction of the acyl chloride with concomitant acetyl migration from the C-1 tertiary hydroxyl to this newly formed primary hydroxyl group. This synthesis ended with the well documented triacetoxy shikimic acid (71).

The second partial synthesis[23] (Fig. 1-21) uses a Hunsdiecker reaction to obtain the triacetoxy-cyclohexanone (45), a key compound which has been referred to twice previously; both in the work of Bestmann and Heid[22] (see Fig. 1-11) and Kitagawa *et al.*[24] (see Fig. 1-12). Grewe's synthesis was not carried beyond the cyclohexene nitrile (72). This method has more recently been used by Corse and Lundin[39] to prepare ^{14}C carboxyl labelled shikimic acid.

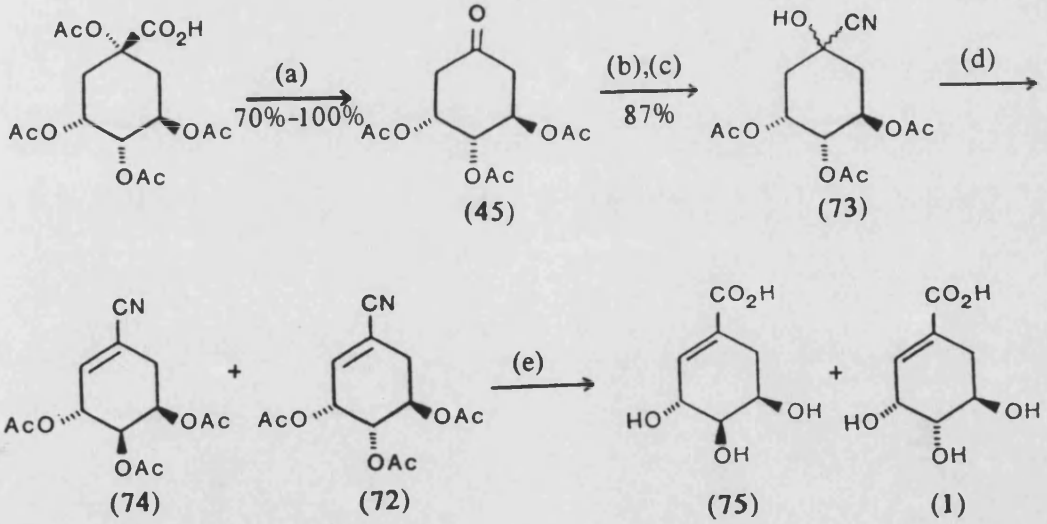


(a) AgOAc, Br_2, EtBr; (b) HCN; (c) $POCl_3$, pyridine.

Fig. 1-21

3. Stereochemistry of Quinate-Shikimate Conversions

Rapoport demonstrated in later work[40] (Fig. 1-22) that dehydration of the cyanohydrin (73) (obtained from nucleophilic addition of cyanide ion to (45)) in fact



(a) AgOAc, Br_2, CCl_4; (b) $NaHSO_3$; (c) HCN; (d) SO_2Cl_2; (e) NaOH; H^+

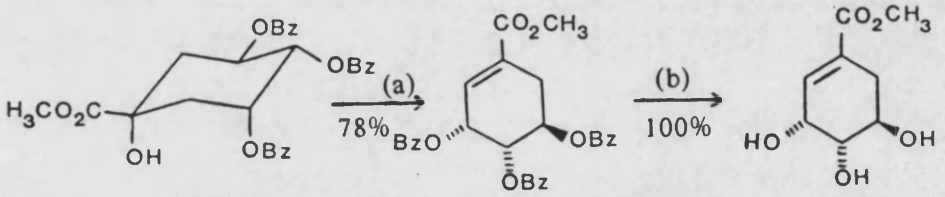
Fig. 1-22

gives two isomeric α,β-unsaturated nitriles (72) and (74). This presence of (74) in the dehydrated mixture was completely overlooked in all the aforementioned syntheses employing (45). It seems that whilst (72) crystallised spontaneously from the reaction mixture (74) was an oil - a fact which probably explains its previous omission.

Separation of (72) and (74) followed by alkaline hydrolysis afforded (-)-shikimic acid (1) and (-)-4-*epi*-shikimic acid (75) respectively.

4. The Synthesis of Cleophax *et al.*

One other synthesis which deserves comment is that of Cleophax *et al.*[41]. These workers selectively benzoylated (-)-methyl quinate, and treated the resulting tribenzoate (76), (Fig. 1-23) with sulphuryl chloride, effecting dehydration to the tribenzoate (77) in 78% yield. Quantitative debenzoylation with sodium methoxide in methanol yielded (-)-(2) in an overall yield of 78%.



(76) (77)

78% overall yield

(a) SO_2Cl_2 pyridine ; (b) NaOMe/MeOH

Fig. 1-23

RESULTS AND DISCUSSION

CHAPTER 2 : APPROACHES TO 6-SUBSTITUTED ANALOGUES OF SHIKIMIC ACID

A. Aims and Objectives

1. Inhibition of Enzymes

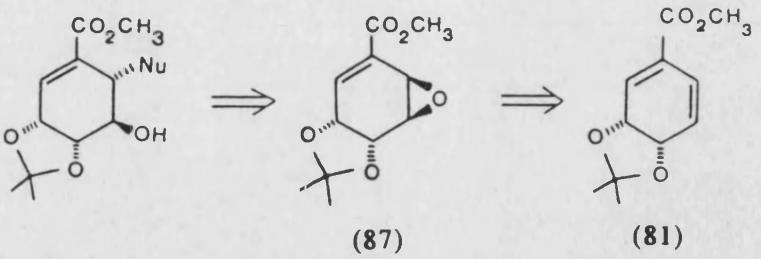
It is well known that analogues of natural products frequently inhibit enzymes, and that this effect is often highly specific. Inhibition[42] can be brought about either by irreversible binding which leads to permanent inactivation of an enzyme, or by a reversible process. In the latter case two possibilities are known : (a) competitive inhibition, where the inhibitor bears a very close structural resemblance to the natural substrate and therefore competes with it for the binding site; (b) non-competitive inhibition. Non-competitive inhibitors bind both to the enzyme-substrate couple as well as to the enzyme itself. Such compounds may not be similar to the natural enzyme substrate, and it seems likely that they react at an alternative or allosteric site on the enzyme surface.

Shikimic acid bearing a substituent at the 6-position isosteric with hydrogen might well function as a competitive inhibitor of the late stages of the shikimic acid pathway, whereas analogues with varying substituents or stereochemistry at the 3-, 4-, or 5- positions might operate in the non-competitive, or even irreversible sense.

In either case the shikimic acid syntheses developed at Bath[43] are ideally suited for the construction of compounds designed to act as enzyme inhibitors.

2. Strategy

The primary objective was to introduce a substituent into the 6- position. It was anticipitated (Fig. 2-1) that this could be accomplished by nucleophilic opening of the epoxide (87) - a compound presumed to be easily obtainable by extension of a previously vindicated synthesis[19] of the diene ester (81).



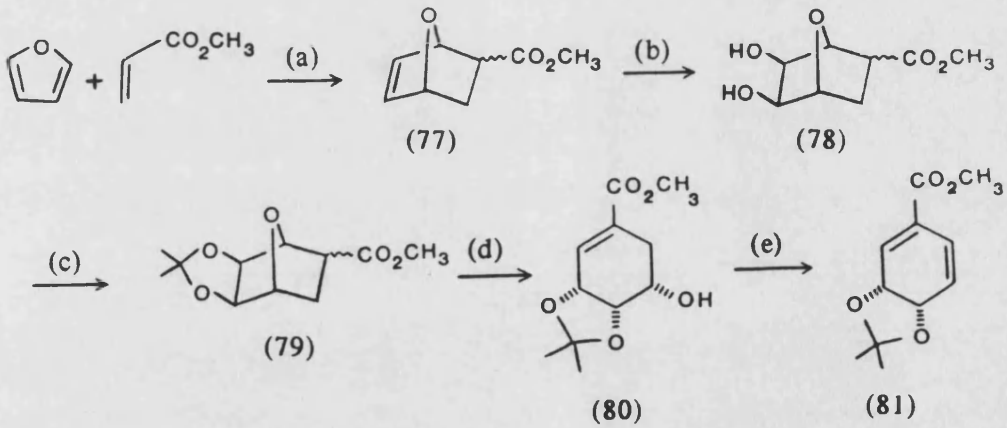
(87) (81)

Fig. 2-1

B. Synthesis of the Diene Ester (81)

1. Diels-Alder Reaction

The first step in this synthesis of diene ester (81) (Fig. 2-2) entailed a zinc iodide catalysed Diels-Alder reaction of furan and methyl acrylate.



(a) ZnI_2, 40°C, 48h.; (b) OsO_4 - *t*-BuOH, H_2O_2, THF, 4 days; (c) $Me_2C(OMe)_2$, *p*-TSA, Me_2CO, 50°C, $CaCl_2$; (d) $[Me_3Si]_2NH$, BuLi, THF, -84°C; (e) DEAD, PPh_3, THF, 1.5h.

Fig. 2-2

Due to its aromatic character furan is a poor diene in Diels-Alder reactions, and only reacts with very good dienophiles under normal conditions of temperature and pressure.

The use of high pressure (≈ 15000 atmospheres) has been found to increase the yields in such difficult reactions[44], but the discovery by Brion[45] that [4+2] cycloaddition of furan and some dienophiles can be greatly accelerated by the addition of zinc iodide means that the adducts (77) can now be obtained with ease, reducing the previously lengthy reaction time of up to two months[46] to a mere 48h. at 40°C. Attempts to use other Lewis acids such as ferric chloride, stannic chloride and zinc chloride have been reported as being much less effective[47].

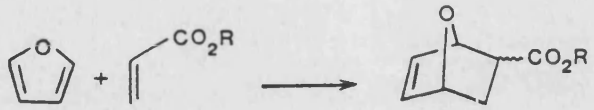
The yield of (77), after distillation, was 32%. This could be increased to 57% if the product was purified by chromatography, but distillation was found to be the quickest and cleanest method, especially for large scale preparations.

An interesting contrast in *endo-exo* selectivity emerges when comparing the uncatalysed, room temperature reaction, reported by Nelson and Allen[46], with our catalysed, 40°C reaction (Table 2-1). The 6:1 *endo* bias quoted for the uncatalysed reaction is, contrary to expectation, reversed in the catalysed reaction, to a 1:3 ratio* favouring the *exo* isomer.

It is generally considered that the effect of adding a Lewis acid catalyst in a Diels-Alder reaction is the formation of a complex with the dienophile[49] rendering it more reactive and more *endo* selective than the uncomplexed dienophile. In our reaction the rate increase was profound: this can not be attributed merely to the elevated temperature since it has been demonstrated (see Table 2-1) that under reflux conditions alone, where ethyl acrylate is the dienophile, the reaction still requires several weeks

* as determined by ^{1}H n.m.r. spectroscopy.

Table 2-1



R	Conditions	*endo/exo* ratio	Reference
CH_3	RT, 2 months	6:1	46
CH_3	ZnI_2, 40°C, 48h.	1:3	This work
C_2H_5	Reflux, several weeks	2:3	48

for completion. However, the putative increase in *endo*-selectivity (brought about by greater secondary interaction of frontier orbitals) did not materialise, and would appear to be more than offset by the formation of the thermodynamically more stable *exo* isomer[50] which predominates at higher temperatures.

Our 1:3 ratio therefore reflects a balance between increasing the *endo* selectivity by Lewis acid catalysis (enhancing the 6:1 ratio) and a greater increase in *exo* selectivity brought about by the increased temperature.

Separation of the adducts (77) was not necessary for our purposes but has been achieved by Nelson and Allen[46], and the isomers were fully characterised. Thus we were able to make our assignements based on data gleaned from this work.

2. Hydroxylation and Ketalisation

Cis-hydroxylation[51] of (77) was accomplished using Milas' reagent - a catalytic amount of osmium tetroxide in the presence of an excess of hydrogen peroxide as the secondary oxidant. The hydroxylation of (77) has been reported as taking ten days to complete[43], but in our hands the *cis*-diols (**78**) were obtained in 65% yield after just four days.

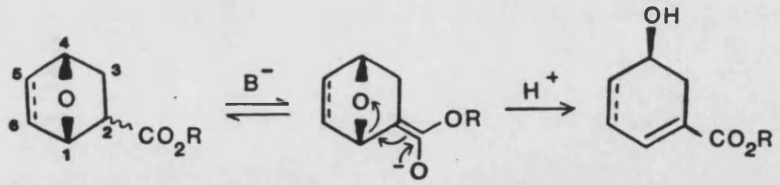
Protection of (78) as the acetonides was completed using a simple acid catalysed exchange reaction with 2,2-dimethoxypropane, this treatment affording (79) in 90% to quantitative yields.

3. Base Mediated Ring Opening

Base induced ring opening of the 7-oxabicyclo [2.2.1] heptane system of (79) was effected cleanly and in high yields (typically 80-90 %) using lithium hexamethyldisilazide (generated by the action of *n*-butyl lithium on 1,1,1,3,3,3-hexamethyldisilizane), producing the hydroxy ester (80).

The conditions for this reaction were discovered by Brion[45] who noted that the choice of base is apparently critical. He claimed that the use of potassium hydride or alkoxides at 50°C effects only retro Diels-Alder reactions, whereas lithium di-isopropylamide (LDA) gives low yields. However, this assertion is not substantiated by Rodrigo *et al.*, who state[52] that both methanolic sodium methoxide and LDA will induce the said ring opening.

Ring scission of 7-oxabicyclo [2.2.1] heptanyl systems (Fig. 2-3) can be considered to be the reverse of a Michael-type reaction, and conforms to a general 5-*endo*-*trig* reversion. As such it is in violation of Baldwin's rules[53] for ring closure.



(82)

Fig. 2-3

However, arguments have been advanced [52b] that the enolate ion (82) formed in this reaction has a large orbital coefficient at C-2 (the HOMO), and, as a consequence of the geometry of the system, this C-2 orbital is correctly aligned to interact with the relatively low lying antibonding orbital (the LUMO) of the C-1-oxygen bond (Fig. 2-4). The result of this interaction is a weakening of the C-1-oxygen bond and the development of the C-1-C-2 π bond as the reaction proceeds. This frontier

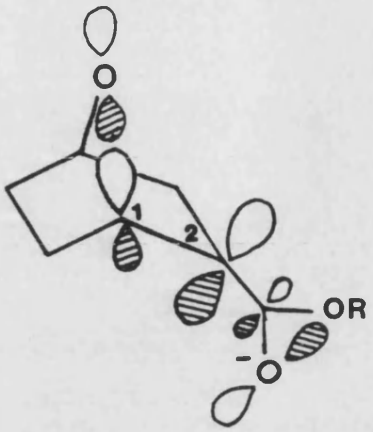


Fig. 2-4

orbital interaction is sufficient to decrease the kinetic barrier to this "forbidden reaction".

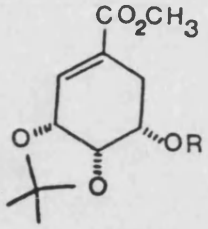
4. Dehydration

(a) The Mitsunobu Reaction

The synthesis was completed by dehydration of the alcohol (**80**) using the so-called Mitsunobu reagent[54] (diethylazodicarboxylate [DEAD] and triphenylphosphine); this mild method afforded the diene ester (**81**) in reasonably good yields (58-74 %) after chromatography.

(b) Other Techniques

Several other dehydration techniques were tried (Table 2-2) using the free alcohol (**80**) as a substrate, and also derivatives of it in which the hydroxyl had been converted to a more efficient leaving group *ie* the tosylate (**83**), mesylate (**84**) and triflate (**85**). However these methods were surprisingly unsuccessful.



(80) R=H
(83) R = $SO_2C_6H_4CH_3$
(84) R = SO_2CH_3
(85) R = SO_2CF_3

Table 2-2. Summary of attempts to dehydrate hydroxy ester (80) and some derivatives of it.

Entry	Substrate	Conditions	Product (yield)
1	(80)	DEAD, PPh_3, THF, R.T., 2.5h.	(81)(58-74 %)
2		$POCl_3$, pyridine, R.T., 5days; 50°C, 24h; DMAP added and reaction stored at 50°C for 3 days[a]	Baseline product-probably phosphate ester
3		$Ph_2S[OCPh(CF_3)_2]_2$, CH_2Cl_2, R.T., 2.5h	(81)(52% corrected yield) + (80)
4		$(F_3CSO_2)_2O$, pyridine[b], CH_2Cl_2, 0°C→R.T., 2h.	(85) (51%)
5		$(F_3CSO_2)_2O$, pyridine, CH_2Cl_2, reflux, 7 days	(85)(43%)+ (81)(26%)
6	(83)	*t*-BuOK, THF, R.T. 48h.	Aromatised
7	(84)	DBU, CH_2Cl_2, R.T., 3h; 40°C, 18h.	No reaction
8		*s*-collidine, $PhCH_3$, 40°C,1h; 70°C,1.5h; reflux,12h.	No reaction

a. A parallel reaction using DBU as the additional base gave similar results.
b. Similar results were obtained using 2,6-lutidine.

Elimination was not achieved from either the mesylate (83) or the tosylate (84), and even when the triflate (85) was formed *in situ* and subjected to comparatively forcing conditions only a small amount of (81) (26%) was obtained.

Since it is possible to construct very reasonable models in which the leaving group and the 6β-H assume an *anti*-periplanar relationship, the reluctance of these compounds to undergo elimination is perplexing, although this may be due partly to an axial effect[55].

However, successful removal of the hydroxyl group was also achieved by using Martin's sulphurane dehydrating reagent (86)[56]. Here, as in the Mitsunobu procedure, the reaction mechanism (Fig. 2-5) involves elimination of a neutral, rather than a negatively charged leaving group. Such reactions appear to work well in difficult cases and are thought to proceed through the E2 mode - the driving force being associated with the formation of the strong S=O (128 kcal mol^{-1})[57] and P=O (130 kcal mol^{-1})[58] bonds.

It is surprising that elimination only occurs to a small extent from the triflate (85) since the trifluoromethanesulphonate ion is an excellent leaving group[59], and normally

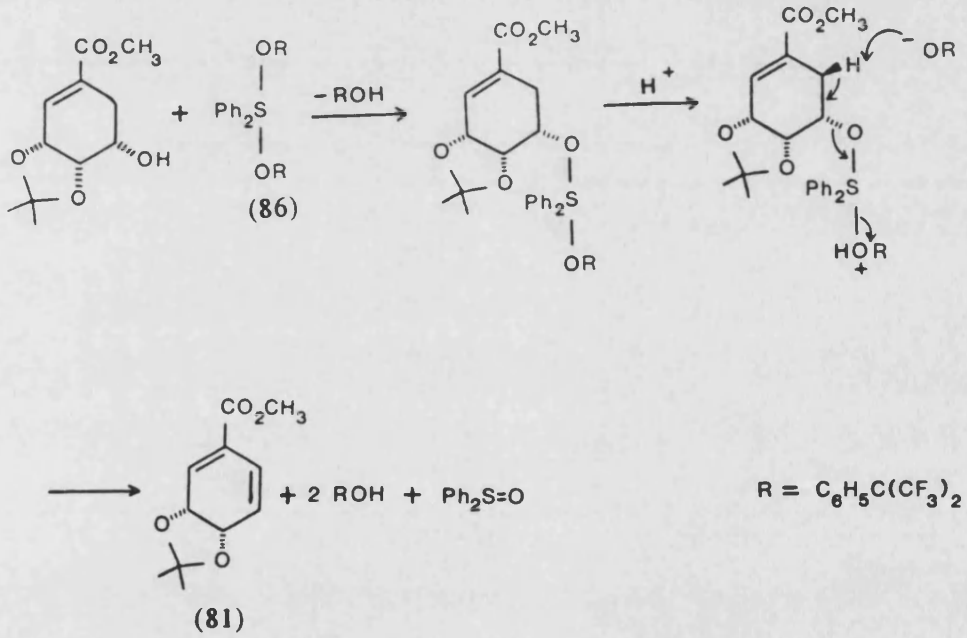


Fig. 2-5

requires only mild conditions for its elimination. The value of the triflate species as a leaving group is clearly demonstrated here since elimination, albeit to a limited extent,

did take place from this derivative, whereas the corresponding mesylate and tosylate failed to react. It is known[60], however, that triflates are some $2x10^4$ to $2x10^5$ times more reactive than the corresponding tosylates.

Although the stability of the triflate (**85**) was unexpected it did facilitate the collection of useful n.m.r. data. Comparing the ^1H n.m.r. spectra of the derivaties (80), (**83**), (**84**) and (**85**), the effect of increasing electron withdrawing power on the chemical shift of the 5-H resonance (Table 2-3) can be ascertained. From a base value of δ 3.95 for the free alcohol the signal appears at increasingly lower field values as the inductive strength increases, culminating in a value of δ 5.11 for the highly electron attracting triflate group. A similar effect is exhibited by the C-5 resonance in the ^{13}C spectra.

Table 2-3

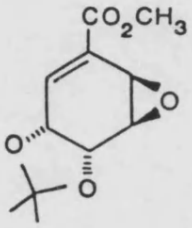
C-5 substituent	δ_H	δ_C
OH	3.95	66.83
OTs	4.77	
OMs	4.94	74.30
OTf	5.11	82.81

C. Epoxidation of the Diene Ester (**81**)

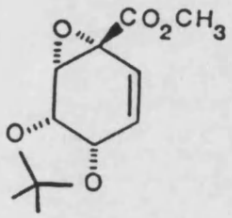
With the diene ester (**81**) in hand it appeared to be simply a matter of oxidising this compound in order to obtain the target epoxide, and we predicted that epoxidation would take place at the most electron rich (*ie* the 5,6) double bond, from the least hindered β-face.

1. With *m*-Chloroperbenzoic Acid

Treatment of the diene (81) with *m*-Chloroperbenzoic acid (*m*-CPBA) in dichloromethane at 40°C gave "one product" (t.l.c.) which was isolated by flash column chromatography. The low resolution ^1H n.m.r. spectrum of this material showed, however, that it consisted of a mixture of two epoxides, (87) and (88), in the ratio of 8:3.



(87)



(88)

Despite numerous variations of solvent systems these components could not be separated satisfactorily either by flash or plate chromatography.

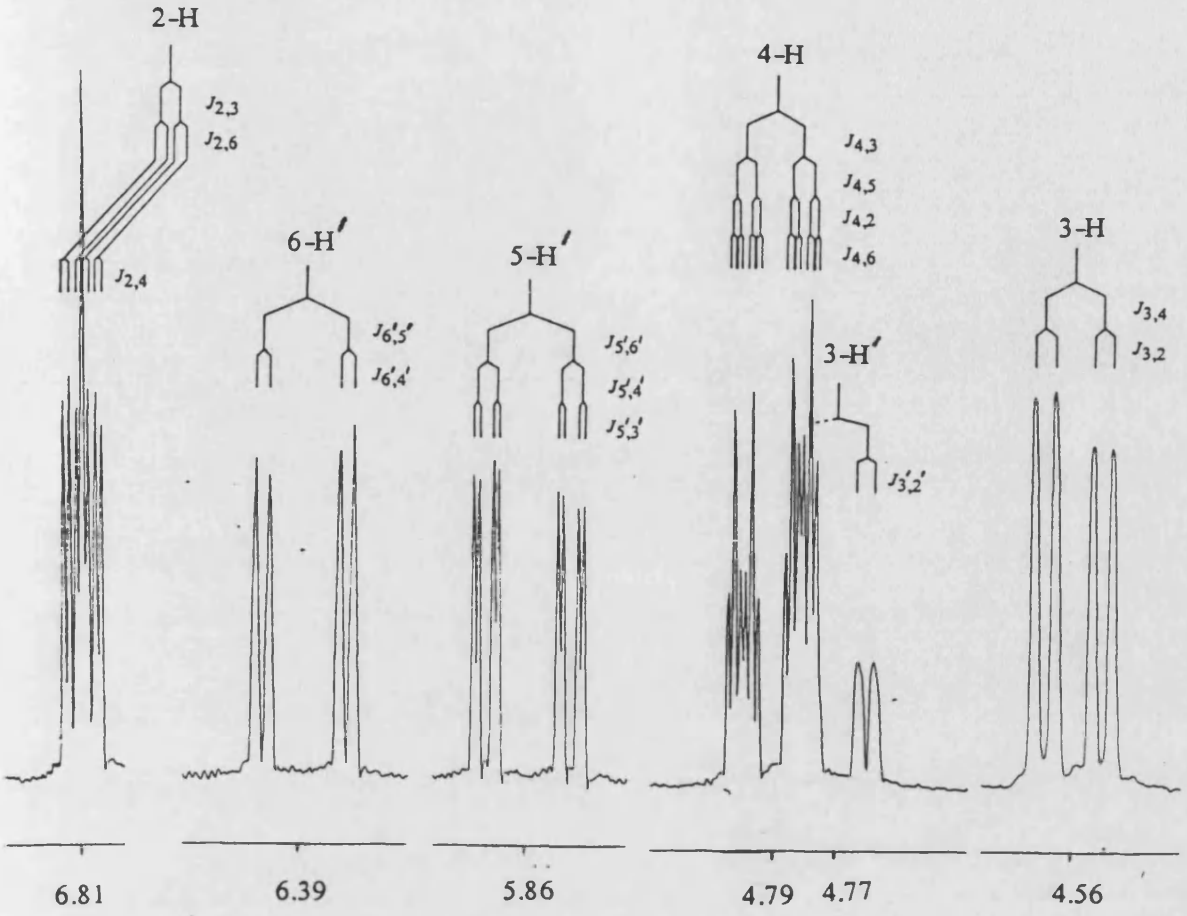
(a) Analysis of the Products

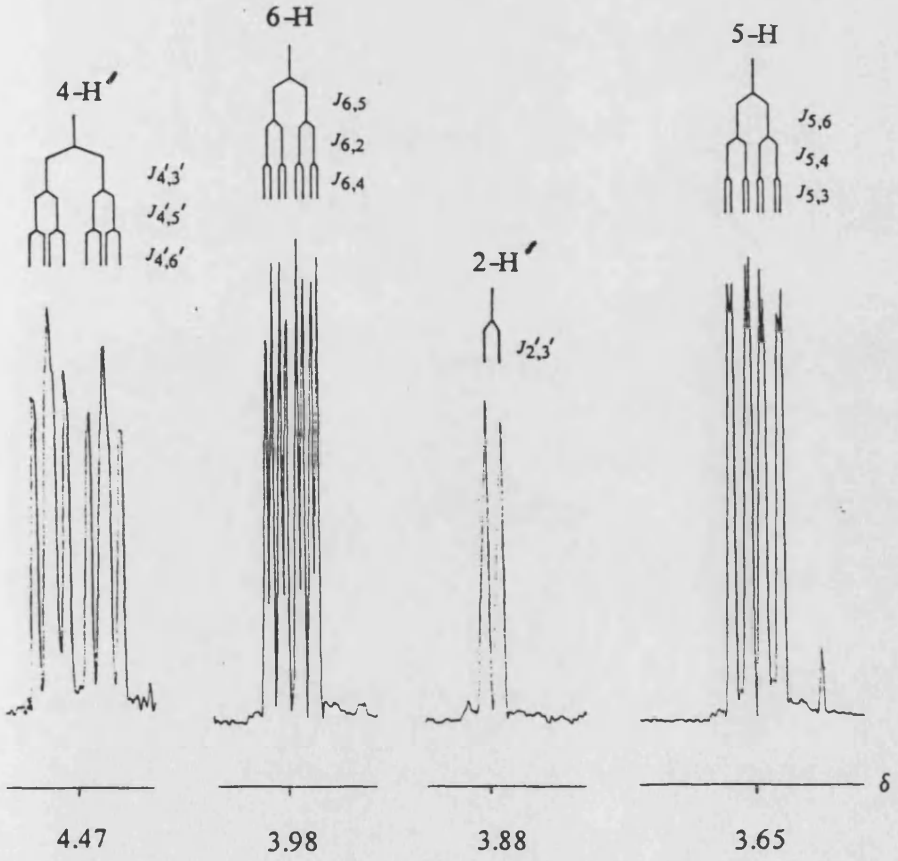
High resolution ^1H n.m.r. techniques enabled a full analysis of the epoxides (87) and (88) to be completed on the mixture. For example, the 400 MHz ^1H n.m.r. spectrum (Fig. 2-6) allowed determination of the regiochemistry of the two isomers. The assignments of signals were made with the aid of 2D COSY spectrum.

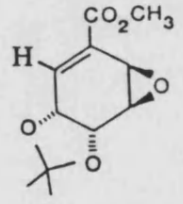
The major product (87) exhibits a low field resonance corresponding to the olefinic proton 2-H. The chemical shift (δ 6.81) is highly characteristic of an olefinic proton in the α,β-unsaturated carbonyl moiety of these systems. This immediately allowed location of the oxirane ring at the 5,6-position. The resonance for 2-H appears as a triplet of doublets: showing vicinal coupling, $J_{2,3}$, of 2.5Hz; allylic coupling, $J_{2,6}$ of 1.5Hz; and ^4J coupling across four single bonds, $J_{2,4}$ of 0.5Hz.

The 3-H (δ4.56) and 4-H (δ4.79) signals are in the expected range for compounds in this series* and also exhibit long range ^4J couplings of 0.5Hz. The resonance due to

* See Appendix I


2-H
$J_{2,3}$
$J_{2,6}$
$J_{2,4}$
6-H′
$J_{6',5'}$
$J_{6',4'}$
5-H′
$J_{5',6'}$
$J_{5',4'}$
$J_{5',3'}$
4-H
$J_{4,3}$
$J_{4,5}$
$J_{4,2}$
$J_{4,6}$
3-H′
$J_{3',2'}$
3-H
$J_{3,4}$
$J_{3,2}$
6.81
6.39
5.86
4.79
4.77
4.56


4-H′
$J_{4',3'}$
$J_{4',5'}$
$J_{4',6'}$
6-H
$J_{6,5}$
$J_{6,2}$
$J_{6,4}$
2-H′
$J_{2',3'}$
5-H
$J_{5,6}$
$J_{5,4}$
$J_{5,3}$
δ
4.47
3.98
3.88
3.65


CO_2CH_3
H
O
O
O

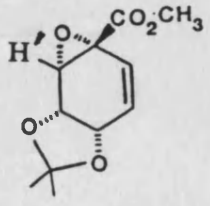
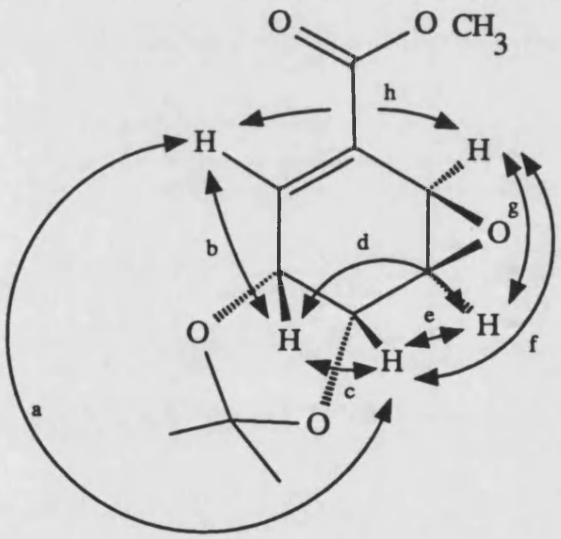

H
O
CO_2CH_3
O
O

Fig. 2-6

5-H (δ3.65) is within the limits normally expected for an epoxidic proton, but that of 6-H is at unusually low field (δ3.98) - a consequence of its allylic nature and its proximity to the methoxycarbonyl group (which exerts both an electron withdrawing and a deshielding influence on 6-H). Couplings for this major isomer are summarised below.

It can be seen that several long range "w-couplings" occur in this compound, and the splitting patterns in this spectrum typify the multiple nature of spin-spin couplings observed in these ring systems.



Ja= 0.5 Hz
Jb= 2.5 Hz
Jc= 7.0 Hz
Jd= 0.5 Hz
Je= 2.0 Hz
Jf= 0.5 Hz
Jg= 3.5 Hz
Jh= 1.5 Hz

Assignments of the resonances observed for the minor isomer (**88**) were also made with the assistance of the 2D COSY technique. The resonances due to 2-H (δ3.88), 3-H (δ4.77) and 4-H (δ4.47) all occur at predictable chemical shifts, but for this compound two olefinic resonances can be seen at δ5.86 and δ6.39, implying that epoxidation has taken place at the "eneone" double bond of the diene (**81**). The 6-H

resonance in this compound, as in (87), is again at lower field due to its juxtaposition with the methoxycarbonyl moiety.

Unfortunately, the coupling constants were of little value in determining the relative stereochemistry of each oxirane ring, but this is hardly surprising since the normal Karplus correlation[61] is unreliable in estimating J_{vic} values between epoxidic

$J = J^{\circ} \cos^2 \Phi - c \qquad [0° \leq \Phi \geq 90°] \qquad J^{\circ} = 8.5Hz \qquad c = -0.3Hz$

$J = J^{180} \cos^2 \Phi - c \qquad [90° \leq \Phi \geq 180°] \qquad J^{180} = 9.5Hz$

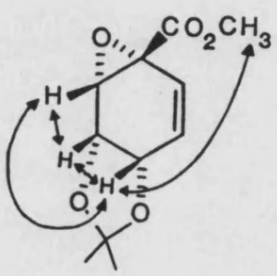
and adjacent protons, and whilst a revised Karplus equation[62]

$J = 5.1 \cos^2 \Phi \qquad [0° \leq \Phi \geq 90°]$

has been proposed, it is not widely applicable.

Assignment of the relative sterochemistry of the epoxide (88) was based primarily on nuclear Overhauser effect difference spectroscopy (NOEDS). This is a double resonance technique which relies, for its effect, on intramolecular relaxation through dipole-dipole interaction. The magnitude of the effect[63] is inversely proportional to r^6 (where r is the intramolecular separation), and for protons, the interacting nuclei should be no more than 3.5Å apart. Although this technique can be used quantitatively to establish distances between nuclei, its' use in organic chemistry is generally restricted to qualitative determinations.

The results which allowed the relative stereochemistry of the epoxide (88) to be assigned are shown below;



⟷ indicates observed nOe

The two key enhancements are those between 4-H and the methyl ester protons, and between 4-H and 2-H. Models show that neither of these would be possible if the epoxide had a β-configuration.

Unfortunately NOEDS could not be used to establish the stereostructure of the isomer (87), since whilst a n.O.e. was observed between 4-H and 5-H, models show that these protons would be in close enough proximity to exhibit this effect regardless of the stereochemistry of the oxirane ring.

(b) Attempts to Separate the Products

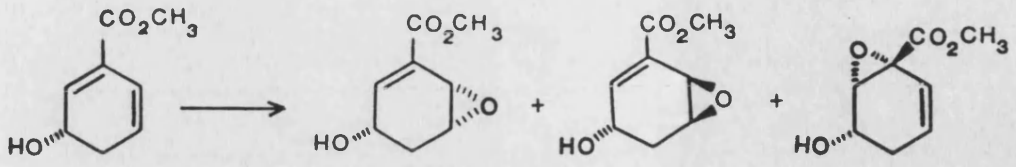
Having obtained a mixture of epoxides (87 and 88) at 40°C using *m*-CPBA, the reaction was repeated at both ambient, and sub-ambient temperatures, in attempts to exploit any differential reactivity that may exist between the two double bonds of the substrate. After extended reaction times the same binary mixture was produced in each case.

Further concerted efforts to separate these isomers included extensive experimentation with numerous solvent systems for t.l.c., gas liquid chromatography* (g.l.c.) and high performance liquid chromatography (h.p.l.c.), but no technique afforded satisfactory separation.

(c) Literature Precedent

Our results are in accord with the problems encountered by Thomas *et al.*[64] in their work on arene hydrates. This group found that treatment of the methyl benzoate hydrate(89) with *m*-CPBA (Fig. 2-7) afforded a mixture of epoxides (90), (91) and (92) in the ratio of 50:15:35 respectively (epoxidation using *t*-butyl hydroperoxide and molybdenum hexacarbonyl gave the same epoxides in a ratio of 65:20:15). They found that the isomers (91) and (92) were inseparable.

*Good resolution was obtained using capillary g.c. but this technique did not translate to the preparative scale.



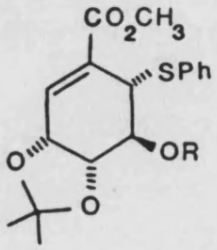
(89) (90) (91) (92)

Fig. 2-7

(d) Experiments to Establish the Stereostructure of the Major Isomer by Reaction of the Mixture with Sodium Thiophenolate

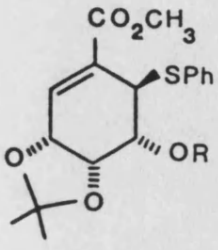
(i) Analysis of the Hydroxysulphides (93) and (94).

In order to ascertain the relative stereochemistry of the major component (87) of the epoxide mixture we took the crude reaction mixture from a large scale experiment, and subjected it to treatment with thiophenolate anion (generated *in situ* from sodium hydride and thiophenol) at 0°C. Two products were isolated in 35% and 20% yields, which, to our surprise, proved to be the epimeric hydroxysulphides (93) and (94) respectively.



(93)R=H

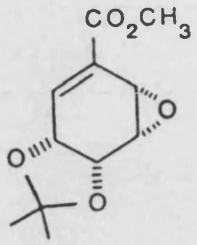
(95)R=Bz



(94)R=H

(96)R=Bz

Clearly the major product (93) was derived from nucleophilic ring opening of the major epoxide (87), but the unexpected product (94) must have arisen from a similar attack on the diastereomeric epoxide (97). The presence of this epoxide was subsequently revealed by h.p.l.c. analysis of the crude epoxide mixture.



(97)

There is absolutely no doubt about the structures of the hydroxysulphides (93) and (94). Exchange experiments allowed recognition of the carbinol resonance in each case, and double resonance experiments identified this as due to 5-proton in both isomers.

High resolution ^1H n.m.r. spectroscopy was employed to accurately determine the coupling constants for each isomer, and once these had been established, the relative stereochemistry of the hydroxyl group at C-5 could be assigned. The amount of data amassed on compounds of this type (for some examples see Appendix I) has established that the magnitude of $J_{4,5}$ is diagnostic in determining the relative stereochemistry of a substituent at the 5-position. If the compound has a 5β-substituent (*ie* the shikimate configuration) then $J_{4,5}$ is large, ranging from 6-9 Hz, whereas for compounds with a 5αsubstituent (*ie* the 5-*epi*-shikimate configuration) $J_{4,5}$ is in the region of 1-5Hz.

The hydroxysulphides (93) and (94) exhibit $J_{4,5}$ couplings of 9.0 and 5.0Hz respectively, and on this basis, their stereostructures were specified (this necessitates the assumption that the epoxides are opened by the normal S_N2 mechanism). Furthermore, by extrapolating back, the relative stereochemistry of the substrates could be deduced as being that of a β-epoxide (87) (major component) and α-epoxide (97) (minor component) respectively.

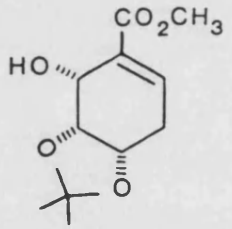
Isomer (97) was not detected in the previous epoxidation mixtures probably because these had been "purified" prior to analysis, but it was observed in this crude reaction

mixture both by h.p.l.c. and high resolution ^1H n.m.r. analysis. Unfortunately the chemical shifts of the proton resonances in this compound were invariably masked by those of the other compounds (87) or (88), making the task of extracting meaningful data impossible.

(ii) The Fate of Compound (88)

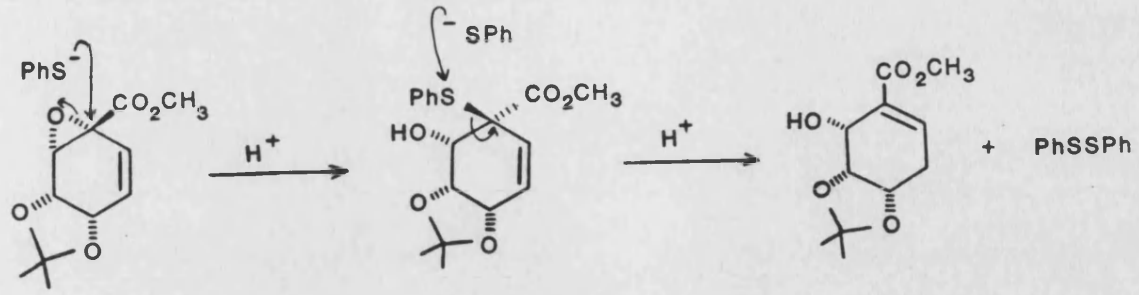
Another surprising result from this reaction was that no product was observed due to the epoxide (88) despite consumption of all the starting material. A separate experiment was conducted to establish its fate.

Treatment of the pure isomer (88) (obtained through a process described later) with sodium thiophenolate in a manner identical to that outlined above, gave only one product in 23% yield - the allylic alcohol (98).



(98)

Treatment of the epoxide (88) with sodium hydride alone induces no reaction *per se*, and this must therefore preclude direct S_N2' opening of the epoxide by hydride ion. This suggests that an addition - elimination sequence operates, such as that outlined in Fig. 2-8. This involves an initial attack by thiophenolate ion at C-1, and protonation to give the intermediate (99). This intermediate might then undergo a nucleophilic attack at the sulphur atom from a second thiophenolate ion to give a stabilised anion, α- to the carbonyl group. On protonation this would yield the allylic alcohol (98) and diphenyl disulphide. There are precedents for this suggestion such as those reported for the attack of mercaptans on diethyl azodicarboxylate[65], and upon thiophthalimides[66].



(99)

Fig. 2-8

(iii) Analysis of the Benzoyl Esters (95) and (96)

Overwhelming proof of the structures of the sulphides (93) and (94) was obtained by converting these compounds into the benzoyl esters (95) and (96). This was achieved for each by stirring the free alcohol with benzoyl chloride in triethylamine.

The ^1H n.m.r. spectrum of the α-benzoate (96) in $CDCl_3$ exhibits resonances

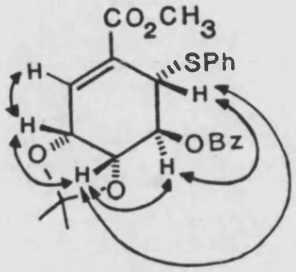
Table 2-4

COMPOUND	SOLVENT	OBSERVED RESONANCES (p.p.m.)				
		2-H	3-H	4-H	5-H	6-H
(96)	$CDCl_3$	6.85	4.86	4.53	6.11	4.22
(95)	$CDCl_3$	7.05	4.87*	4.87*	5.22	4.87*
(95)	$CDCl_3/C_6D_6$	6.96	4.42	5.00	5.56	5.18

*These resonances appear together as a multiplet centred at 4.87 p.p.m.

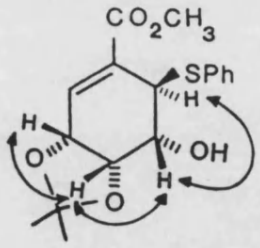
which occur at predictable chemical shifts for all of the ring protons (Table 2-4). Conversely the ^1H n.m.r. spectrum of the β-benzoate (95) in $CDCl_3$ exhibits second order behaviour, with the chemical shifts of 3-, 4- and 6-H signals all down field with respect to the corresponding resonances of the parent compound (93). These

signals occur together as a multiplet centred at δ4.87. The addition of C_6D_6 to the sample caused these overlapping signals to occur at different chemical shifts, and allowed a series of NOEDS experiments to be carried out on the isomer (95). The most significant results are shown superimposed in the structure below:



⟷ indicates observed n.O.e.

The most important outcome of these experiments is the observation of a n.O.e. between the resonances due to 4-H and 6-H. These protons are in close proximity in the *pseudo*-chair conformation of the compound where both 5- and 6- substituents occupy equatorial sites. The n.O.e. could not possibly occur if the configurations at C-5 and C-6 were reversed, so that the phenylthio group assumes a 6β configuration. This fact is further supported by NOEDS data acquired for the hydroxysulphide (94):



⟷ indicates observed n.O.e.

In this case there is no enhancement effect from the resonances of 4-H to 6-H.

It will also be noticed that a significant n.O.e. is observed between 4-H and 5-H of compound (94) where these protons are *cis*, whereas only a very small enhancement (see experimental) is observed between 4-H and 5-H of the phenylsulphide (95), where the protons are *trans*.

2. Alternative Epoxidation Conditions

Having rigorously established the structures of the major products of *m*-CPBA epoxidation of diene ester (81) as the epoxides (87) and (88) (and also established the existence of the isomeric epoxide (97)), and been unsuccessful in extensive attempts to separate these compounds, we next employed a number of different epoxidation techniques, in an effort to maximise the proportion of the isomer (87) produced - to the exclusion of the alternative compound (88), if possible.

For example, use of the less reactive monoperphthalic acid[67] in ether increased the reaction time, but still gave the same two component mixture (34%), whereas trifluoroperacetic acid[68], caused rapid aromatisation of the substrate. Attempts to moderate the latter reaction by repeating it at low temperature, and in the presence of potassium carbonate or phosphate buffer had no effect.

Traditionally epoxides are synthesised by treating an olefin with "HOBr" and reacting the intermediate bromohydrin with a base.[69] However, when the diene (81) was subjected to this procedure it was found to yield a multi-component mixture, and it would appear that bromohydrin formation was not selective.

Epoxidation with the sterically demanding vanadium (IV) 2,4- pentadionate oxide *t*-butyl hydroperoxide system[70] also gave the now familiar binary mixture of epoxides (87) and (88).

Moving away from the more conventional methods of epoxidation we then used Payne's reagent[71] (perbenzimidic acid), noted for its sometimes unusual stereochemical preferences[72].

The reagent was generated in the reaction medium, using Gagnieu's modification of Payne's original procedure[73], from benzonitrile and hydrogen peroxide in the presence of potassium carbonate as a basic catalyst. After 2.5h. under these conditions the sole reaction product, isolated in quantitative yield, was the epoxide (88).

A summary of the epoxidation reactions employed is given in Fig. 2-9.

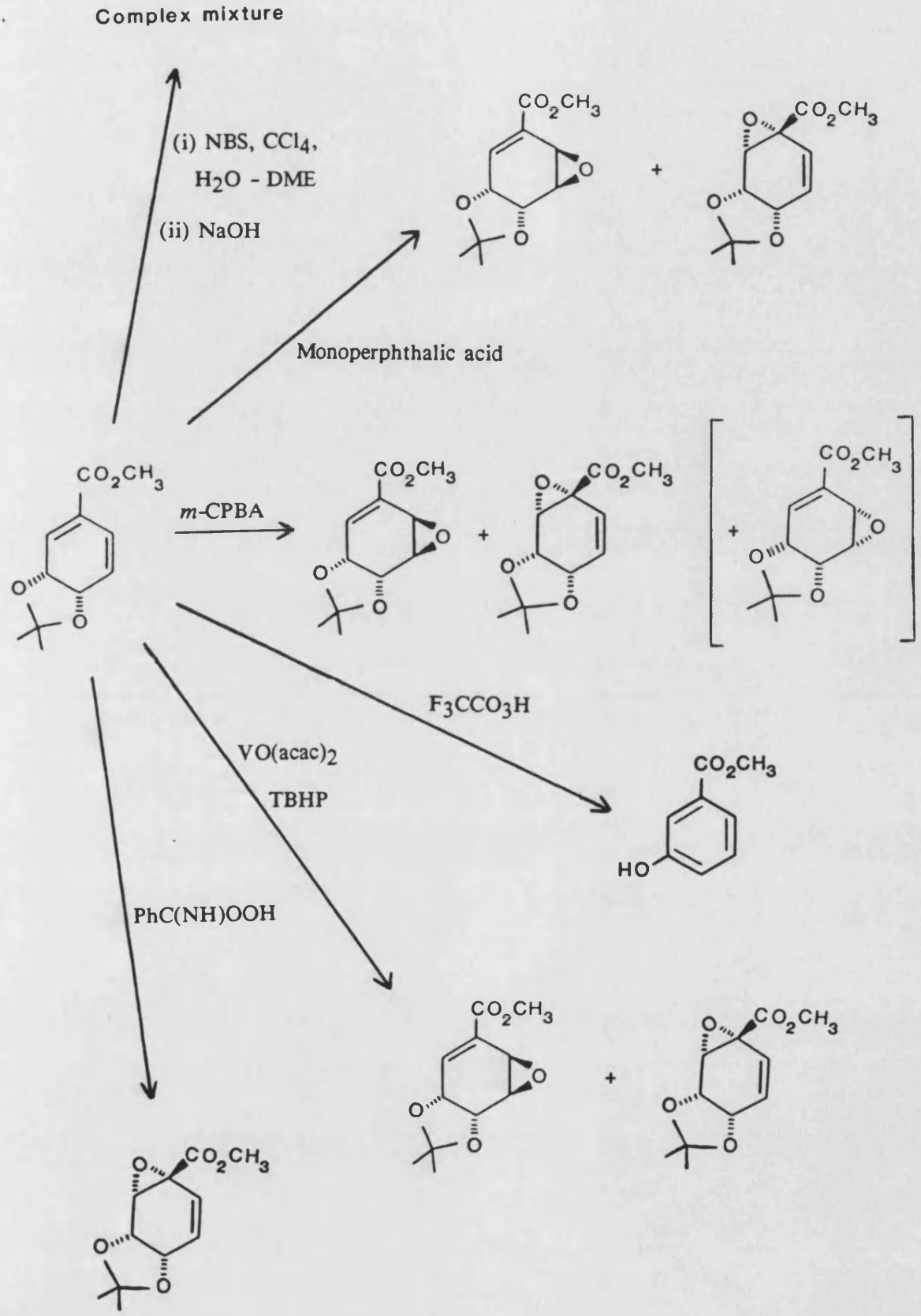


Fig. 2-9 Summary of Epoxidation Reactions of (81)

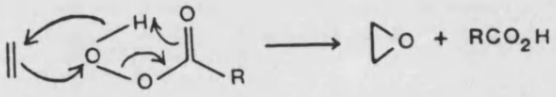
3. Proposed Reaction Mechanisms

The question therefore arises as to why the reaction of the diene (81) with peracids such as *m*-CPBA gives a mixture of 1,2α- and 5,6β- epoxides, whereas treatment with perbenzimidic acid yields the 1,2α-epoxide exclusively.

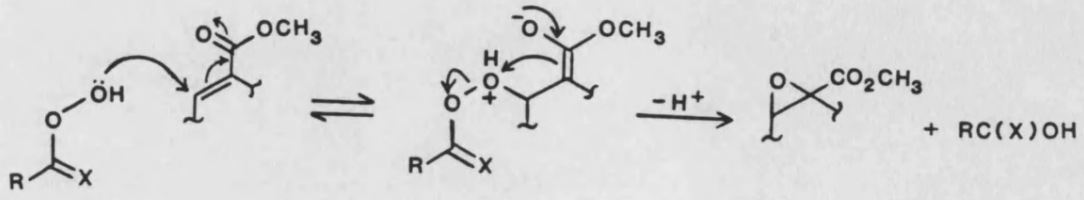
Two factors need consideration if an explanation is to be advanced : the regiochemistry; and the stereochemistry of the epoxides formed in each reaction.

In order to rationalise the observed regiochemistry it is proposed that epoxidation of the 1,2-double bond occurs by a different reaction mechanism to that operating to give the 5,6-epoxide.

It is suggested that epoxidation of the more electron rich 5,6-double bond takes place by the usual electrophilic Prilezhaev reaction, the generally accepted mechanism[74] for which, (in vague terms), is shown below:

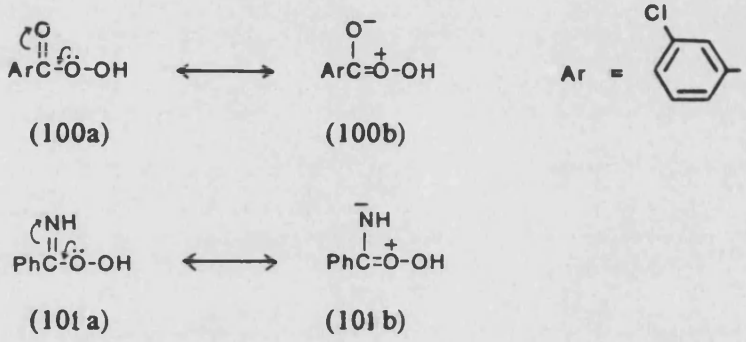


Conversely, epoxidation of the 1,2-double bond may proceed by an initial nucleophilic attack on the α,β-unsaturated ester, in a Michael-like fashion thus:



m-CPBA X=0 , Perbenzimidic acid X=NH

A precedent for this proposal can be found in the epoxidation of α,β-unsaturated carbonyl compounds with alkaline hydrogen peroxide (the Weitz-Scheffer reaction) which is said to operate by a nucleophilic addition[75]. It is also recognised[76] that epoxidation of a polar double bond by a weak peracid can be regarded as being an intermediate case between direct electrophilic attack and this type of conjugate addition.



m-CPBA(100) is more electrophilic than perbenzimidic acid (101), consequence of the greater electron withdrawal induced by the carbonyl group of the former reagent. Since the carbonyl oxygen of (100) is more electronegative than the imidic nitrogen of (101), a greater development of positive charge is induced at the central carbon atom of the former (100).

A consequence of this is that the more nucleophilic perbenzimidic acid will attack the diene ester (81) exclusively in the Michael-like fashion, whereas the more electrophilic *m*-CPBA operates *primarily via* the Prilezhaev mode, but functions in the alternative, nucleophilic sense, to a limited extent (*ie* in attacking the 1,2-double bond).

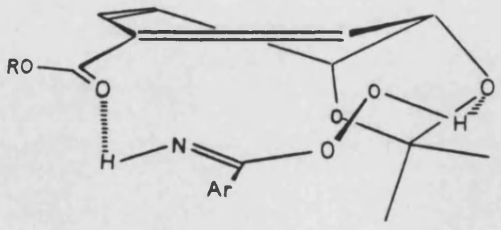
It can be seen that the nucleophilic epoxidation pathway is many times faster than electrophilic epoxidation since the 1,2-epoxide (88) is formed from (81) within 2.5h. at 0°C with perbenzimidic acid, whereas a reaction time of 12h. at 40°C is necessary to produce the 5,6-epoxide (87) plus some of the 1,2-isomer (88).

The proportion of 1,2-epoxide (88) obtained with *m*-CPBA is commensurate with the low nucleophilicity of this peracid and the faster reaction rate of nucleophilic

epoxidation.

The β-orientation of the predominant 5,6-epoxide (**87**) was predicted, and can be accounted for by assuming that electrophilic attack by the peracid occurs from the least hindered β-face of the diene (**81**). Epoxidation of the 1,2-double bond would also be expected to occur from the least hindered β-face, but in fact the α-epoxide is formed at this position. This suggests that the peracid is directed onto the more hindered α-face by association with the lone pair of electrons of the ether oxygen atom at C-3 of the substrate. Molecular models confirm the feasibility of this type of reagent approach control and also suggest a second possible hydrogen bonding site when perbenzimidic acid is the reagent (Fig. 2-10). In this case a two-membered encounter complex would be formed which would deliver the nucleophilic oxygen onto the α-face at the correct angle of attack.

Henbest[77] has noted that directive effects, frequently observed during the attack of an electrophilic species on cycloalkenes, can result from dipole-dipole interactions between a remote substituent and the olefin-peracid transition state. But whilst this reasoning may be readily employed to explain the β-stereochemistry observed in the epoxide (**87**), it cannot account for the α-isomer (**88**), and may not be widely applicable for multisubstituted compounds.

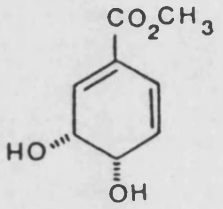


⋯⋯ Indicates H-bonding

Fig. 2-10

D. Synthesis and Epoxidation of the Diol (102)

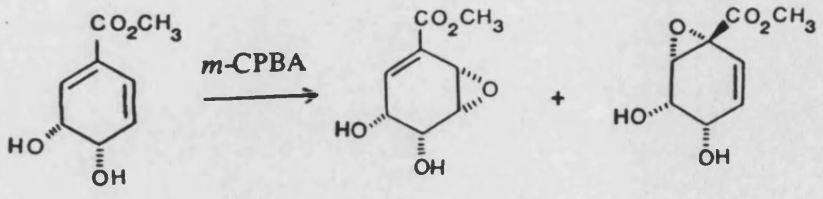
It was clear by now that there was little chance of obtaining the 5,6-isomer regioselectively by epoxidation of the diene (81), and so a simple peracid epoxidation was carried out on the deprotected compound (102), to perceive if any variation in selectivity could be conferred. The diol (102) was prepared[43a] in 86% yield from the



(102)

protected diene (81), by simply heating at 56°C with 50% aqueous acetic acid for 1.5h.

The products obtained from epoxidation of the known compound (102) with *m*-CPBA (Fig. 2-11) were found, by analysis of the ^1H n.m.r. spectrum, to be a 1:1 mixture of the regioisomeric epoxides (103) and (104). These were both assigned α-stereochemistry in accordance with the well documented fact that allylic alcohols direct peracid epoxidation to the *syn* face of a double bond[78], even in cases where this is



(102) (103) (104)

Fig. 2-11

normally difficult. Such directional effects involve a hydrogen bonded transition state (Fig. 2-12) which also has a rate promoting effect. This is exemplified by the epoxidation of the substrate (**102**), which requires a reaction time of only 12h at ambient temperature, compared with the three days at ambient temperature required for epoxidation of the diene (**81**).

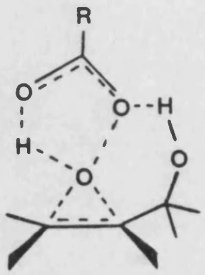
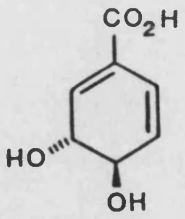


Fig. 2-12

1. Related Literature Compounds

The diol (**102**) is a known compound[19] and is structurally similar to the diol acid (**105**) synthesised by Gibson *et al.*[79], by treatment of chorismic acid with dilute hydrochloric acid. The same compound has also been obtained by Berchtold *et al.*[80] and was found to be biosynthesised from chorismic acid by the organism *Aerobacter aerogenes*[81].



(**105**)

E. <u>Alternative Approaches to Epoxide 87</u>

At this point it was decided to adopt a different approach to the problem of synthesising the epoxide (**87**). Two routes were designed to overcome the problem of poor selectivity observed in the epoxidation of the diene ester (**81**).

The two variants, outlined in retro synthesis below (Fig. 2-13) both begin with the hydroxy ester (**80**), and both envoke a 1,4-addition of thiophenol to the "enone" bond, which would, in effect, protect this 1,2-bond. Thiophenol was chosen because of its dual capacity to act both as a good nucleophile in the 1,4-addition reaction, and as a good leaving group when in a higher oxidation state. The routes converge with the olefinic phenylsulphide (**106**), and it was envisaged that this compound would undergo

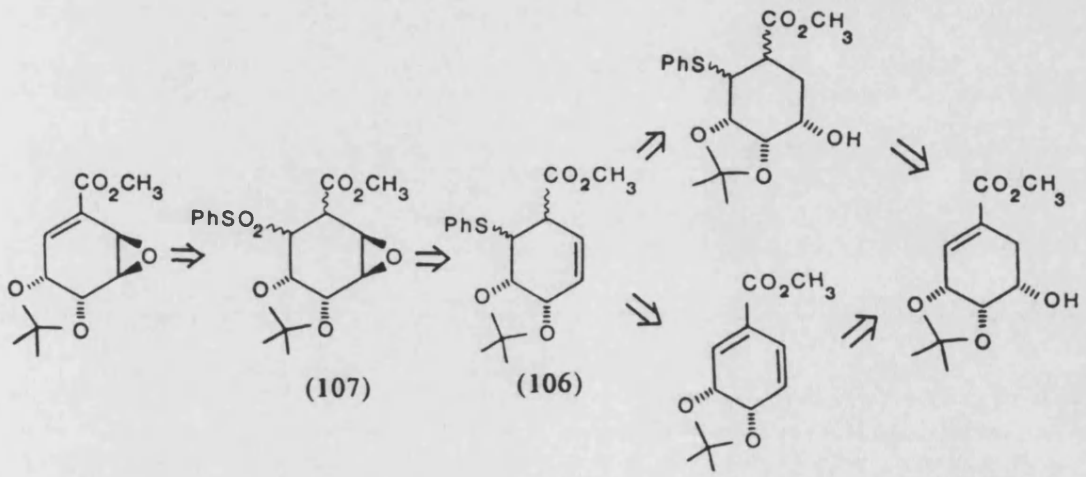
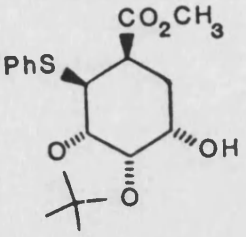


Fig. 2-13

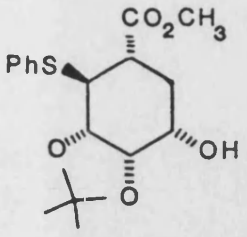
epoxidation with concomitant oxidation of the sulphide to the sulphone when treated with three equivalents of a peracid. With the phenylsulphonyl moiety of epoxide (107) now acting as a leaving group, a facile elimination was anticipated upon treatment of this compound with base. This would reconstitute the 1,2-double bond and reveal the desired epoxide (**87**).

1. Route 1

Reaction of hydroxy ester (**80**) with sodium thiophenolate (pre-formed from thiophenol and sodium hydride at 0°C) progressed slowly at ambient temperature (18h) to furnish two adducts (**108**) and (**109**) (corrected yields 50% and 23% respectively) together with unreacted starting material.



(108)



(109)

The major adduct (108) could be obtained in a pure form after flash chromatography, but the minor product (109) had a R_F coincidental with that of the starting material in a variety of solvent systems, and could not be isolated free from it.

The stereostructure of (108) was assigned by analysis of the coupling constants of the ^1H n.m.r. spectrum, and NOEDS data. It should be noted that the values acquired for coupling constants represent a weighed average[82] of all the conformations a molecule adopts, since the energy barrier between the alternative forms is small enough to permit rapid interconversion at the temperature of the experiment*. This dynamic equilibrium which exists in solution epitomises the behaviour of compounds reported in this thesis - a factor which necessitates caution when interpreting the ^1H n.m.r. data.

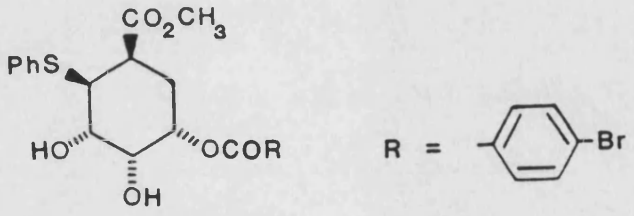
The medium coupling of 4.5 Hz for $J_{1,2}$ is a typical value for protons bearing an axial-equatorial relationship, and models show that this is the case for 1-H and 2-H in all conformations of (108). The larger 6 Hz coupling for $J_{2,3}$ is marginally below the value expected for a diaxial arrangement of protons, and so must encompass a significant contribution from conformers in which 2-H and 3-H are forced into a diequatorial arrangement.

* At temperatures below the point at which ring reversal coalesces in the n.m.r. experiment, the conformational preferences of cyclohexenes have been found to be strongly dependent on solvent polarity[83].

This assignment was strengthened by NOEDS results: irradiation of 2-H resulted in enhancements of the signals corresponding to 1-H (21%) and 3-H (5%). The large discrepancy in these enhancements indicates that the average separation of 1-H and 2-H is much smaller than that of 2-H and 3-H. It was concluded from this that the 1- and 2- protons are *cis* to one another, whereas the 2- and 3- protons bear a *trans* relationship (a conformer where the 2- and 3- protons are *trans* diaxial cannot exhibit a significant enhancement effect).

Taking all these data into account we were able to determine the relative stereochemistry of the alcohol (**108**).

It was desirable to confirm these results by converting this compound into its *p*-bromobenzoate ester, but under the reaction conditions (stirring the alcohol with bromobenzoyl chloride and triethylamine in dichloromethane for five days at ambient temperature) the isopropylidine protecting group was also removed. Nevertheless the product (**110**) formed "good quality" crystals which were submitted for x-ray analysis.



(110)

The results, illustrated in Fig. 2-14 (bond lengths and bond angles are given in Appendix II) substantiated the stereostructure we had proposed for the compound, and encouraged us to feel confident in our ^1H n.m.r. based assignments of other structures in this series.

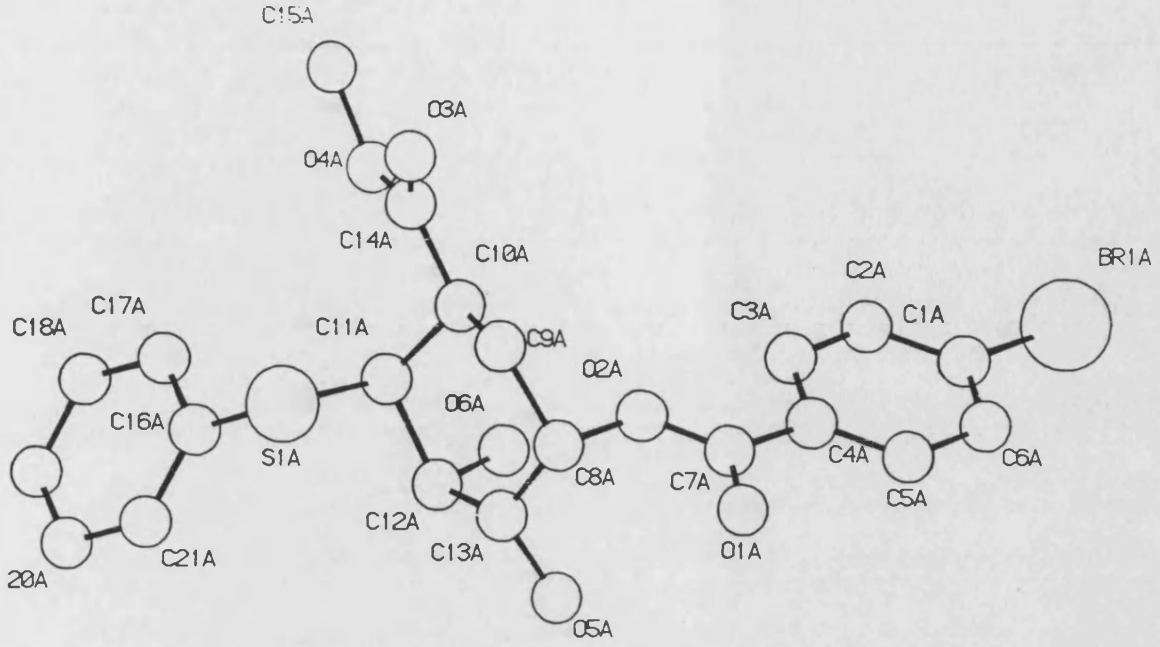


Fig. 2-14 X-ray Crystallographic Determination of (110)

The relative stereochemistry of the minor adduct (109) was confidently assigned, based solely on ^1H n.m.r. coupling constants. The large couplings of 11.5 Hz and 9.0 Hz for $J_{1,2}$ and $J_{2,3}$ respectively, can clearly be reconciled only with a *trans* diaxial arrangement of these protons. Hence the structure shown for (109) is the only alternative.

From these results it is evident that the thiophenolate anion attacks the hydroxyester (80) in a Michael fashion from the more accessible β-face, to give the intermediate (111) (Fig. 2-15).

Protonation of this intermediate at C-1 can take place either from the more easily accessible α-face to give the *cis* product (108), or, alternatively from the β-face which yields the *trans* isomer (109). The preferred chair conformation of (108) would appear to be where the 1,2 and 5 substituents are all equatorial (cf. (108) where at least one of these three substituents must be axial), but, despite this apparently more favourable orientation pattern, it is the *cis* adduct which predominates.

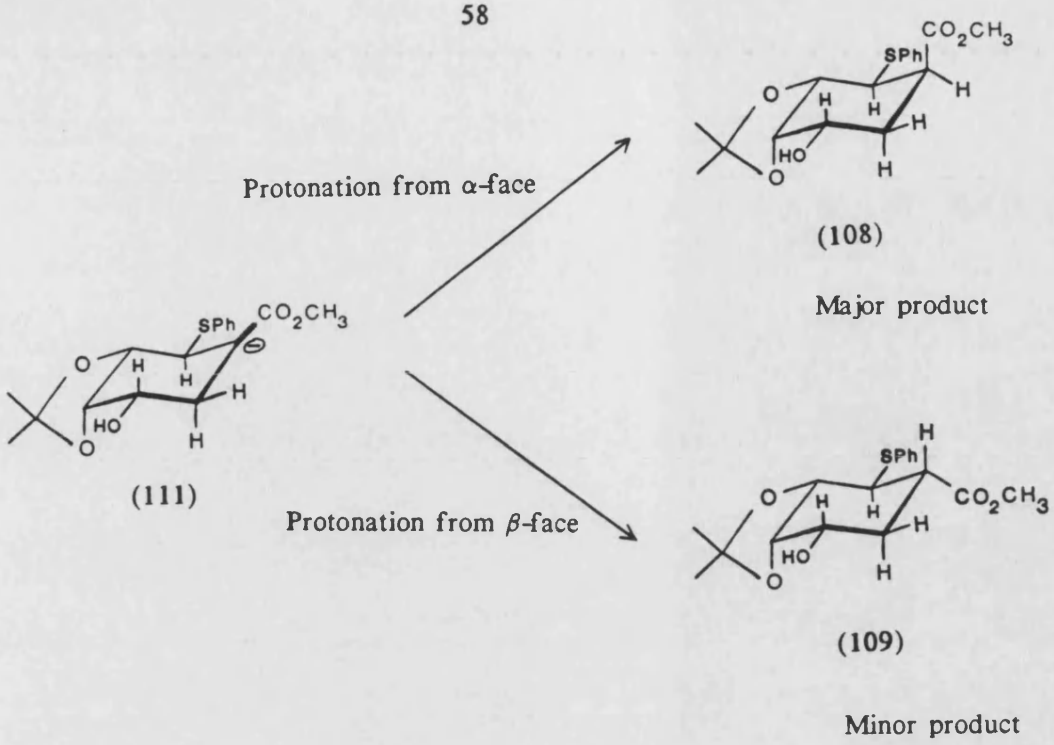
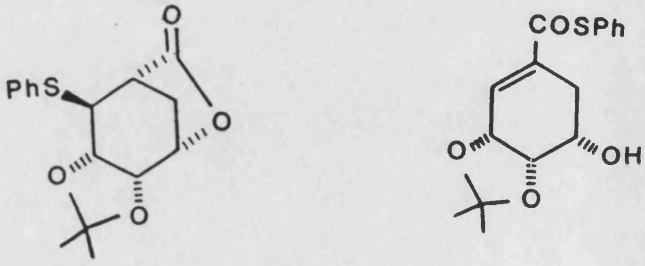


Fig. 2-15

When the reaction was carried out on a larger scale it was overtly slower (four days) and gave a dramatically reduced yield. Moreover, in addition to the expected *cis*-adduct (108) (9%) and *trans*-adduct (109) (isolated with some starting material, 5%), the major product recovered from this reaction, in 12% yield, was the lactone (112).

An analysis of the ^{1}H n.m.r. spectrum appeared initially to be ambiguous, suggesting either the lactone (112) or the thiol ester (113) as possible structural alternatives. However, no change in the spectrum was apparent on deuteration, ruling out the isomer (113). The infrared spectrum was also useful for diagnostic purposes



(112) (113)

(showing a carbonyl stretching at band 1760 cm^{-1}, characteristic of γ lactones), as was

the ^{13}C n.m.r. spectrum, in which an olefinic carbon resonance was notably absent.

The stereostructure of the isomer (112) was deduced entirely by ^{1}H n.m.r. coupling constants. The small couplings between 1-H and 2-H (2.5Hz) and 2-H and 3-H (1.0 Hz) imply diequatorial arrangements between both sets of protons. Now clearly lactonisation can only occur when the C-1 substituent is α-orientated, and since the stereochemistry of the C-3 substituent has been arbitrarily designated α, it follows that the phenylthio group at C-2 must adopt the β-stereochemistry.

We were again proved correct by an X-ray crystallographic analysis of compound (112), which confirmed our initial assignment (Fig. 2-16).

Since the phenylthio group is *exo* (or β) in this compound it would appear that a thiophenolate ion attacks the hydroxyester (80) from the β-face, as before, to give the intermediate (111), and that lactonisation occurs subsequent to this.

An impasse which rendered this route impractical came with unsuccessful attempts to dehydrate the adducts (108) and (109) to obtain the alkene (106). Initially dehydration was tried on the mixture, and then later on the single isomer (108), using the Mitsunobu conditions. None were successful and the starting materials were recovered in all cases. Other reagent systems were also employed, but in each case the starting material was either recovered or destroyed without any evidence of dehydration having occurred. Consequently attention was focused on the alternative route.

A Summary of the progress of route 1 is given in Fig. 2-17.

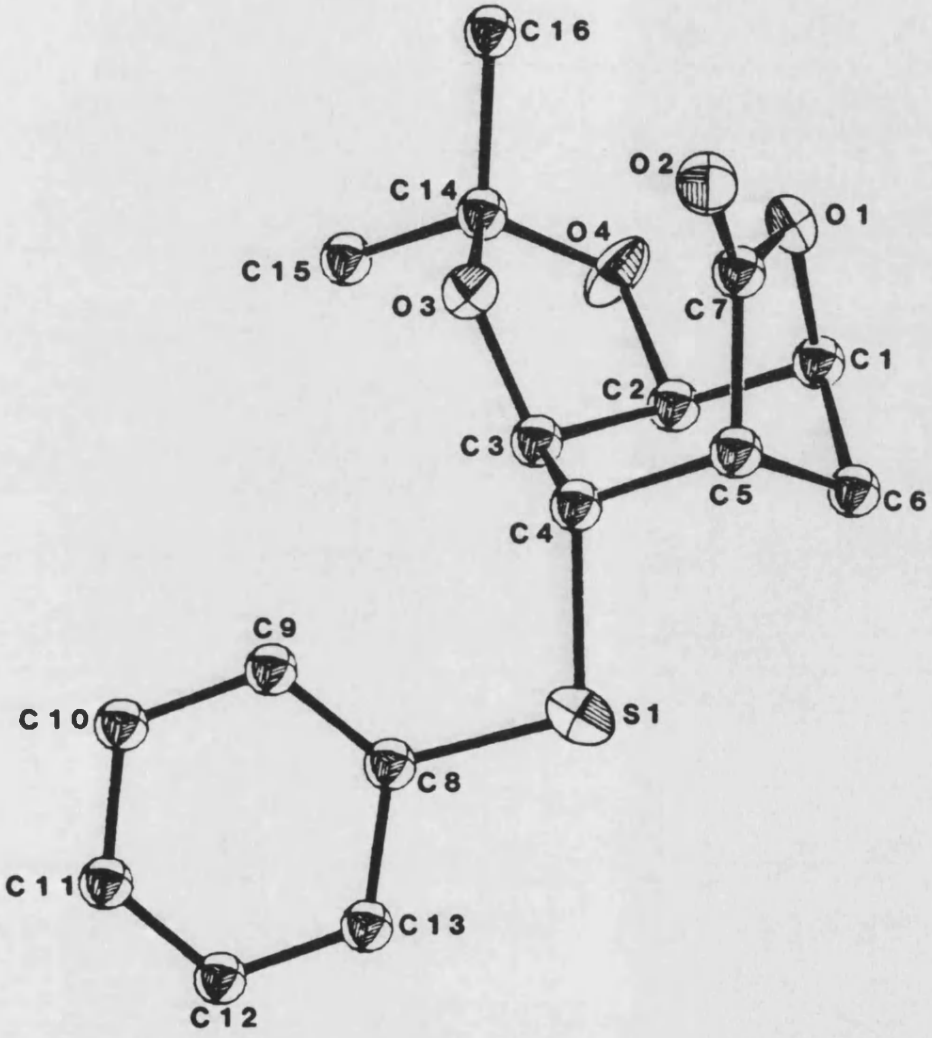


Fig. 2-16

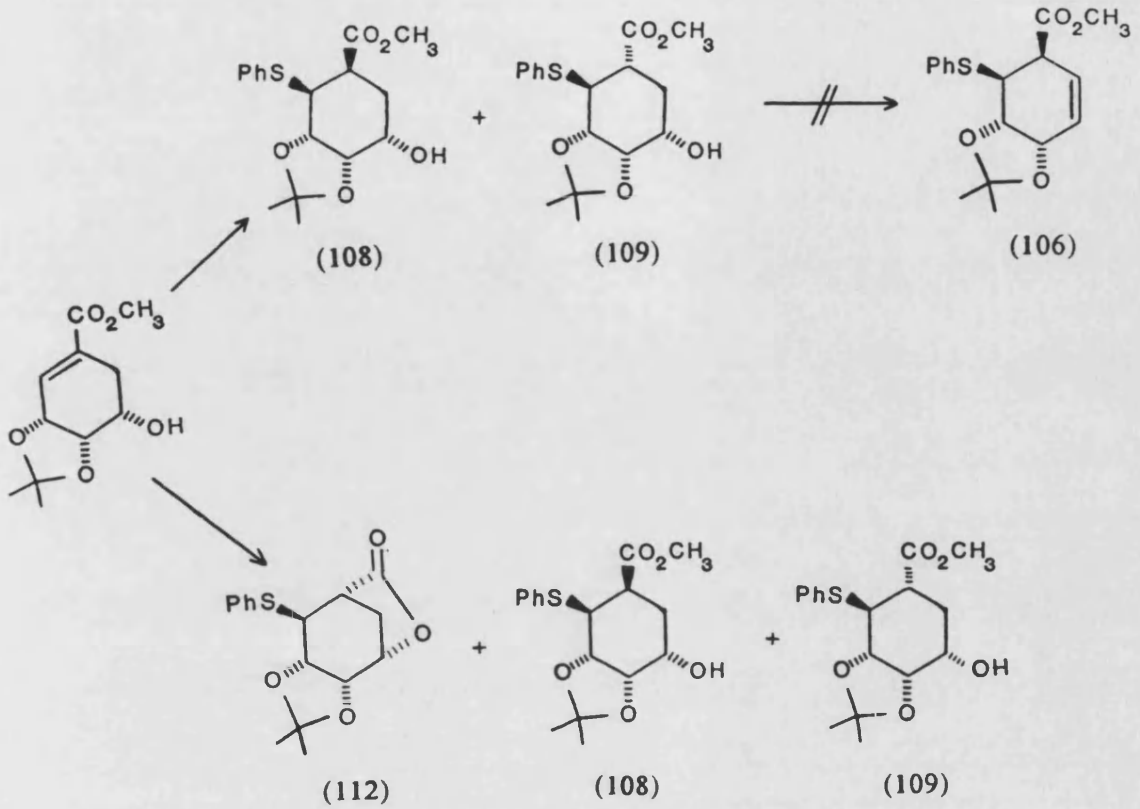
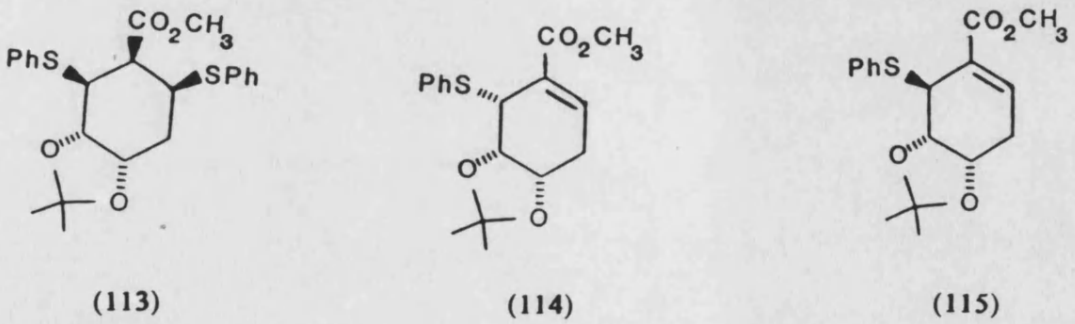


Fig. 2-17

2. Route 2

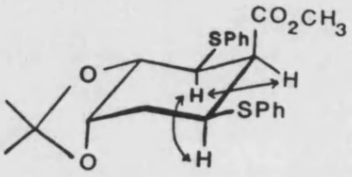
A solution of the diene ester (80) in THF was treated with sodium thiophenolate at 0°C with the intention of forming the olefinic phenylsulphide (106) directly. However, the unexpected product which prevailed in this reaction was identified by low resolution ^{1}H n.m.r. spectroscopy as the disulphide (113). This compound was



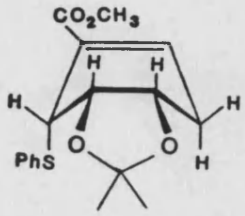
isolated by flash chromatography in 45% yield, and its relative stereochemistry elucidated by high resolution n.m.r. experiments.

Again ^1H n.m.r. coupling constants and NOEDS data provided the basis for the stereochemical assignment. The large $J_{2,3}$ coupling of 9.5 Hz can only be reconciled with a *trans* diaxial arrangement of the protons, establishing the 2-substituent as β and The smaller couplings between 1-H and 2-H (4.5 Hz), and 1-H and 6-H (4.0 Hz), are indicative of axial-equatorial or di equatorial relationships.

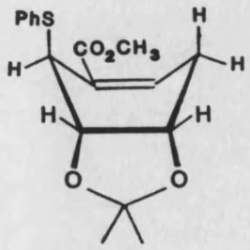
It was therefore possible to use the 2α-H as a reference point in NOEDS experiments. Thus upon irradiation of the 2-H signal a n.O.e. was observed to both 1-H and 6-H, indicating quite clearly the *syn* relationship of these protons:



By means of very careful chromatography two further products were isolated - the α-, and β- allylic sulphides (114) and (115), respectively. Only boat conformations are possible for (114) and (115), and ^1H n.m.r. indicates that for these compounds the preferred boat conformations occur



(114)

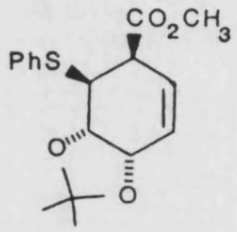


(115)

Fig. 2-18

where the phenylthio groups are axial (Fig. 2-18), since these presumably avoid unfavourable interactions between the 2 and 6 protons which would occur in the alternative boat conformers. There are no discernable "averaging effects" in the coupling constants, so it can be assumed that the equilibrium for ring reversal lies conspicuously towards the preferred boat conformations of (**114**) and (**115**).

The reaction conditions were modified by using triethylamine to catalyse the addition of thiophenol [84] to the substrate (**81**), whereupon the reaction proceeded smoothly to give the desired alkene (**106**) in 59% yield as a colourless, crystalline solid. Once again sound stereochemical determination was based on ^{1}H n.m.r. coupling constants and was verified by NOEDS.



(**106**)

The value of 5.5Hz for $J_{1,2}$ suggests a *cis* arrangement of the C-1 and C-2 substituents since this value is typical of coupling constants between an axial and an equatorial proton. Had a *trans* arrangement existed between the substituents then they might reasonably be expected to exist in a predominantly diequatorial arrangement, in which case the $J_{1,2}$ value (a weighted average) would be higher due to a greater contribution from the *trans* diaxial protons. This situation has been observed for other compounds of this series (cf. structure (**109**)).

This evidence for the *cis* arrangement is further supported by a large reciprocal n.O.e. observed between 1-H and 2-H. NOEDS allowed these protons to be to more accurately defined as having a β relative stereochemistry. Irradiation of the 2-H signal gave a large n.O.e. enhancement (18%) for 1-H and a relatively small enhancement for the 3-H/4-H* signal. These results are compatible with a *cis-trans* relationship between

* These signals occurred together in the 400 MHz ^{1}H n.m.r. spectrum.

the protons 2,1 and 2,3. Irradiation of the 1-H signal gave a large enhancement at the 2-H signal but, significantly, no enhancement of the 3-H/4-H resonance. Such an enchancement would almost certainly have been present if the C-1 methoxycarbonyl group had been α-orientated and the C-1 proton β. In this case 1-H would be in close enough proximity to 3-H and 4-H to show a strong mutual interaction.

Furthermore, the ^1H n.m.r. coupling constants and n0e enhancements recorded for this compound compare very favourably with those of the adduct (**108**) for which the relative stereochemistry is irrefutable.

It appears highly likely that the adduct (**106**) is also the initial product in the reaction of sodium hydride-thiophenol with substrate (**81**), but that under the reaction conditions base induced isomerisation to the allylic sulphide (**115**) takes place. This product is then susceptible to a further 1,4-addition of a thiophenolate ion.

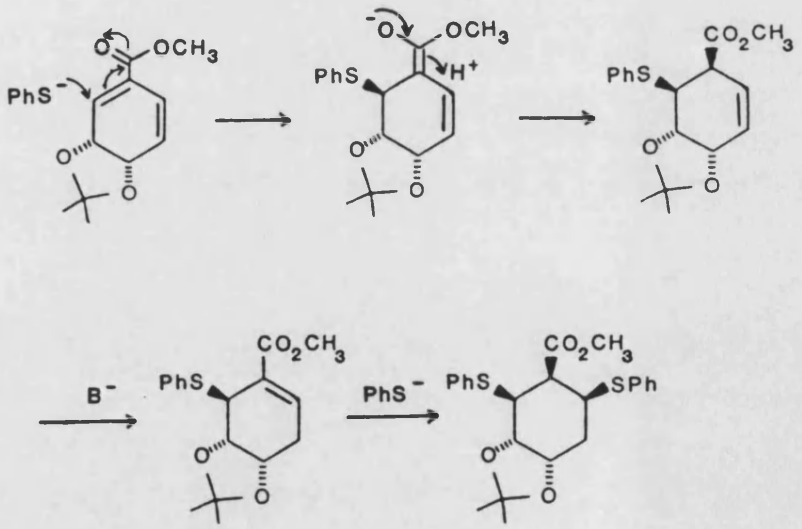


Fig. 2-19

Table 2-5

Entry	Reaction Time (mins)	Product Ratio as % Age of Reaction Mixture		
		(114)	(115)	(113)
1	*ca* 5	15	76	9
2	60	6	10	84

Table 2-5 shows the ratios of α- : β- : di- sulphides after the reaction mixture was quenched almost immediately (entry 1), and when it was allowed to run "to completion" (entry 2). These results give credence to our proposed mechanism since it can be seen that the ratio of monosulphides (91%) to disulphide (9%) is high after the rapid quench suggesting that these are formed at an early stage in the reaction. As the reaction proceeds then the amount of disulphide becomes proportionally higher (84%) as the monosulphides are consumed (16% at completion).

One can further conclude from these ratios that the reaction of α,β- unsaturated ester (**115**) with thiophenolate is much more rapid than reaction of the corresponding allylic sulphide (**114**), which indeed seems to react no further - no trace of any disulphide of different stereochemistry was found.

Further evidence implicating the adduct (**106**) in this reaction sequence was obtained by subjecting a solution of this compound to the same sodium hydride - thiophenol treatment described above, whereupon isomerisation to the allylic sulphide (**115**) took place (Fig. 2-20). Traces of the sulphides (**114**) and (**113**) were also detected in the product mixture.

Comparison of the pK_a values for triethylamine (10.88)[85] and thiophenolate ion (7.76)[86] show that triethylamine is the stronger base, and so the double bond of the alkene (**106**) might well be expected to isomerise under both sets of reaction conditions. The reason that no isomerisation occurs with Et_3N is due entirely to the low concentration of base in this instance. Since a catalytic amount of triethylamine is used, this means that only a small concentration of thiophenolate anion is present at any given time, and since the 1,4- addition reaction to the diene ester (**81**) is extremely

facile then comsumption of any thiophenolate by this process is rapid. Consequently there is no free anion available to act as a base.

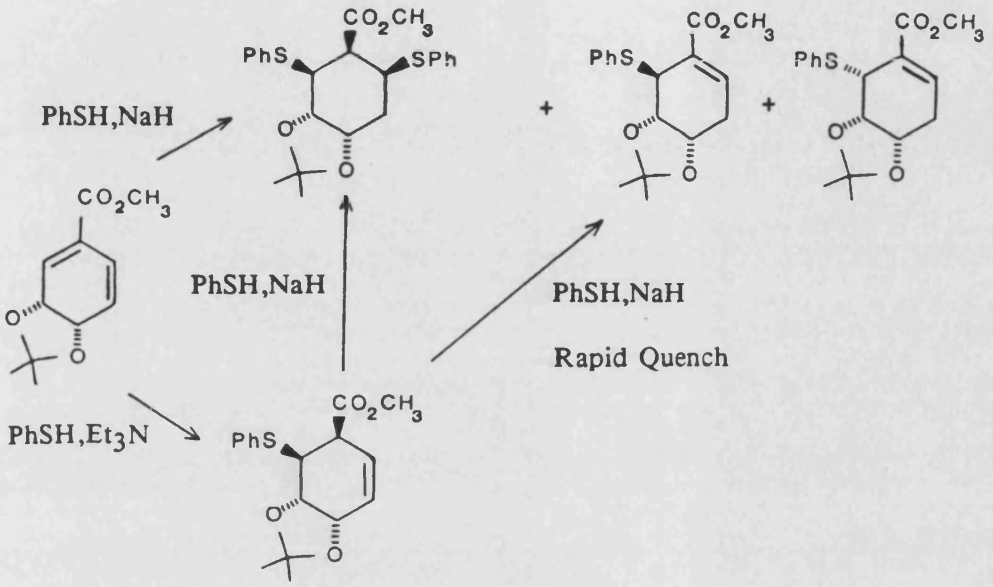
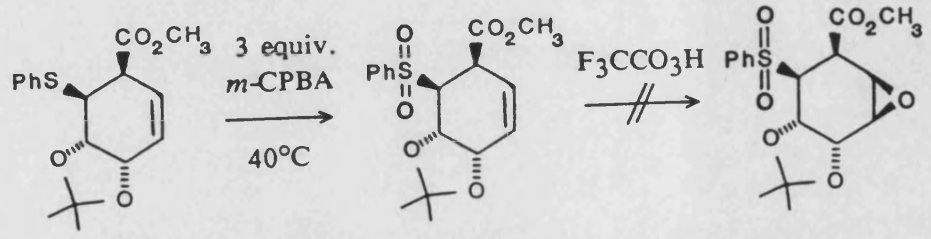


Fig. 2-20

With compound (106) now in hand it appeared a straightforward task to convert this compound to the desired epoxide (107). Treatment with three equivalents of *m*-CPBA at ambient temperature merely succeeded in oxidising the sulphur (Fig. 2-21), affording sulphone (116) in 89% yield, and even under conditions of prolonged heating at 40°C with *m*-CPBA no further reaction took place.



(106) (116)

Fig. 2-21

The sulphone (**116**) was isolated and treated with unbuffered trifluoroperacetic acid, but even these forcing conditions could not induce epoxidation, and the starting material was returned unchanged.

The ^1H n.m.r. spectrum of the sulphone (**116**) showed signals which due to their broadened nature could not be obviously assigned. However these were resolved with the aid of a 2D-COSY spectrum.

The oxidation state of the sulphur in this compound was immediately apparent from the infrared spectrum - the intense diagnostic SO_2 symmetric stretching band at 1150cm^{-1} and asymmetric stretching band at 1310cm^{-1} (*cf.* 1070-1030cm^{-1} for sulphoxides) were both present.

With both these alternative means (Routes 1 & 2) of synthesising compound (**87**) now thwarted, and a physical separation of the epoxides (**87**) and (**88**) not forthcoming, it was necessary to reconsider the approach. Reflecting on the proposed mechanisms by which *m*-CPBA attacks the diene ester (**81**), it was reasoned that the inherent propensity of the "enone" bond to undergo 1,4-addition with the peracid would be lost if the methyl ester of (**81**) could be converted into the corresponding carboxylic acid. This would have the effect of preventing reaction at the 1,2-bond by the Michael-like mechanism and allowing regioselective epoxidation of the 5,6-bond. If this were the case then an added advantage obtained would be verification of the proposed epoxidation mechanisms.

F Mechanistic Approach to Epoxide (87)

1. De-esterification of Diene Ester (81)

(a) Chemical Methods

The diene ester (**81**) was subjected to a variety of de-esterification conditions (summarised in Table 2-6) before a suitable method was found.

The hydrolysis of alkyl carboxylic esters is commonly carried out under acidic or basic conditions. Clearly an acidic hydrolysis was undesirable for this compound

Table 2-6

Reaction conditions	Product	Ref
2M NaOH, MeOH	Aromatic	87
5≡ LiOH, MeOH-H_2O(3:1)	Aromatic	87
TMSI	Complex mixture	88,89
KOH, dioxan	Aromatic	87
PLE, H_2O-Me_2CO(10:1) pH7	(117) > 90%	90

since acid deprotection of the ketal would inevitably lead to rapid aromatisation. The basic conditions employed also caused aromatisation, and so a mild alternative method was sought which would proceed under neutral conditions.

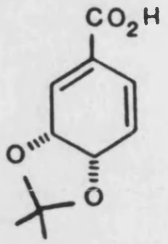
Treatment of an ester with trimethylsilyl iodide is reported to be an efficient alternative to the above methods[88]. Unfortunately both this method and a variation wherein the trimethylsilyl iodide was generated *in situ*[89] gave complex mixtures. This is probably due to competing reations[91] such as deprotection of the ketal and/or nucleophilic attack of iodide ion at the 1,2-double bond.

(b) Enzymic Hydrolysis

Ester hydrolysis was, however, achieved by an enzymic method[92]. Pig or porcine liver esterase is a highly active serine hydrolase. Early studies [93] of the properties and kinetics pointed to the possible existence of more than one esteratic enzyme. Later studies [94,95] revealed these enzyme preparations to be a mixture of at least seven very similar, but chemically different enzymes (known as isoenzymes) which have differing substrate specificites. Furthermore each enzyme has two types of active site. Even highly purified enzyme preparations exhibit some heterogeneity[94a], and this may well explain the differences in the reaction times observed in our own experiments.

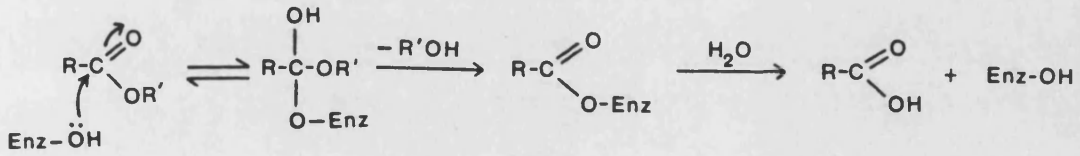
This enzymatic hydrolysis is particularly mild since the reaction can be maintained at pH7 by the periodic addition of phosphate buffer. Hydrolysis of (81) proceeded smoothly (reaction time being governed by the ambient temperature, and by the amount and batch of PLE used) to furnish the diene acid (117) in high yield

(typically >95%).



(117)

The reaction is thought to proceed by a double displacement mechanism (Fig. 2-22) involving an acyl-enzyme intermediate (118)[95a,96]. Initial attack is from the hydroxyl group of a serine residue (hence the classification of PLE as a serine hydrolase). Loss of methanol (R′ = CH_3) gives the acyl-enzyme intermediate (118) which undergoes attack from a second nucleophile, in this case water, to displace the enzyme.



(118)

Fig. 2-22

(c) Kinetic Study

The standard work-up for this type of reaction[90,97] is to acidify the solution and extract the product into an organic solvent. The concern here was that acidification in this case might effect ketal deprotection with consequent aromatisation, and so in order to investigate the acid lability of this diene acid (117) a limited kinetic study was initiated. The work was extended to include the base lability of (117) (which had been observed previously) and also to encompass comparative studies on the diene ester (81).

These unrefined experiments, summarised in Fig. 2-23 fulfilled their primary purpose of establishing that acidification and extraction of the diene acid (117) from the enzymic solution is indeed possible without the problems of deprotection and aromatisation. Although deprotection is the faster reaction, it is not rapid at pH_2-the acidity at which extraction is carried out - and successful extraction was achieved furnishing the diene acid (117)in high yields.

An interesting feature which also arose from these studies is the aromatisation of both substrates (81) and (117) to hydroxybenzoic acids upon treatment with base. Since ketals are resistant to base hydrolysis the aromatisation cannot have involved a diol intermediate. A probable mechanism (Fig. 2-24) involves removal of the 3-proton by base with acetone acting as a leaving group.

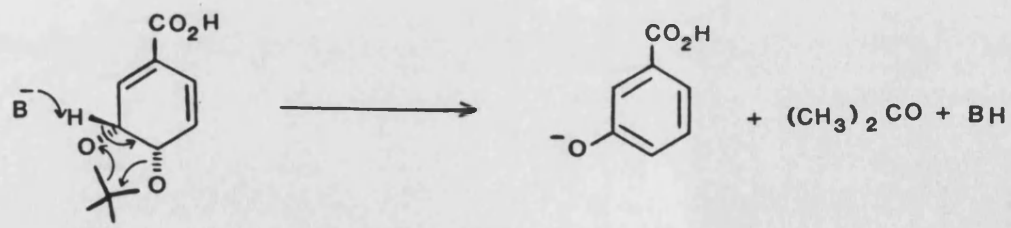
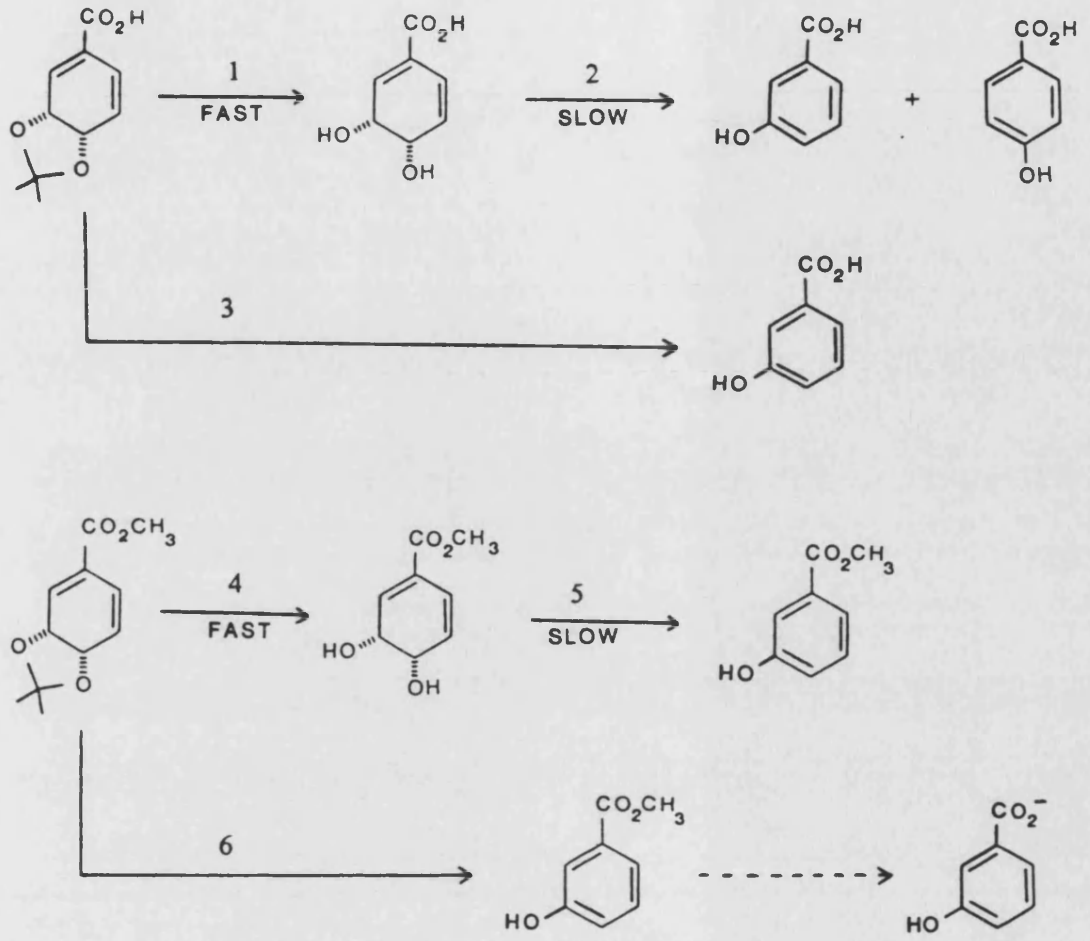


Fig. 2-24



REACTION	REAGENT/ CONC.	pH	METHOD	≈k(min^{-1})	≈$t_{\frac{1}{2}}$(mins)	S.D.*
1.	0.1M $HClO_4$	1	h.p.l.c.	$2.5x10^{-2}$	28	6%
2.	1M $HClO_4$	0	u.v.	$6.4x10^{-2}$	11	0.8%
3.	1M NaOH	13.7	u.v.	$2.1x10^{-2}$	34	0.3%
4.	0.5M $HClO_4$	0.3	h.p.l.c.	$5.7x10^{-2}$	11	8%
5.	1M $HClO_4$	0	u.v.	$6.5x10^{-2}$	11	0.5%
6.	1M NaOH	13.7	u.v.	$1.7x10^{-2}$	42	2%

* It is customary to quote standard deviation as a measure of error, however, this error is outweighed by experimental error and so the S.D. is not a true reflection of the accuracy of the experiments.

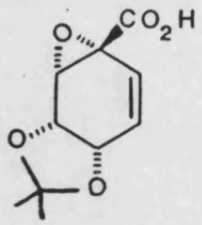
Fig. 2-23

(d) Use of Esterases in Chiral Syntheses

The utility of enzymes as chiral catalysts for asymmetric syntheses is well documented[98]. However, the enantioselectivity of most esterases is only moderate and frequently a large number of enzymes must be appraised before a suitable system is found. In theory though, the separation of enantiomers is possible if the enzyme displays high enantioselectivity, and even if the particular enzyme has only low enantioselectivity a separation may still be possible by kinetic resolution[99].

Since the hydrolysis of the diene ester (**81**) with PLE gave such high yields of the corresponding acid (< 90%) the enzyme clearly did not show high enantioselection with this substrate. The reaction was stopped at various stages of completion in subsequent reactions but no optical activity was detected in the product in every case, so a kinetic resolution was dismissed. A similar situation occured when the esterase α-chymotrypsin was employed.

Of the compounds treated with PLE in this manner only the epoxy ester (88) reacted enantiospecificially. The reaction was sluggish and after three days only a very small amount of product had been formed (19% corrected yield). However, the unreacted starting material was optically active and so too, by implication, was the product-epoxy acid (**119**).

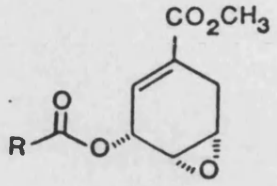


(119)

Work by Sicsic *et al.* on 7-oxabicyclo [2.2.1] heptene systems has shown that the PLE hydrolysis of adducts bearing an *exo*-ethoxycarbonyl substituent is many times faster than that of the corresponding *endo* isomers, enabling separation of *exo-endo* mixtures on a preparative scale. This is in keeping with the findings in the Bath

laboratories[101] for adducts bearing methoxycarbonyl substituents.

More recently Berchtold *et al.* have reported that treatment of the epoxide (63) with PLE results in hydrolysis of the methyl ester in preference to the side chain ester, with no kinetic resolution observed. In experiments with lipases and cholesterol esterases



(63)

the side chain ester was preferentially hydrolysed with an accompanying kinectic resolution.

This lack of enantioselectivity shown by PLE substantiates our findings, but with the large number of hydrolytic enzymes now commercially available, and the option of changing the ester group in our compounds, there exists a clear possibility that the syntheses reported in this thesis could be made chiral.

2. Epoxidation of the Diene Acid (117)

The diene acid (117) was treated with *m*-CPBA in dichloromethane at ambient temperature, and this successfully accomplished a regio- and stereoselective epoxidation. For characterisation and ease of handling the epoxy acid (120) was converted directly into the known epoxy ester (87) by treatment with diazomethane[102] (Fig. 2-25).

A sample of the fully deprotected compound (121) was prepared by treating the epoxide (120) with strongly acidic ion exchange resin[103] for the purposes of biological testing.

Esterification therefore allowed confirmation of the relative stereochemistry of the epoxide formed. The treatment of compound (120) with diazomethane had to be carefully monitored since an excess of the reagent afforded a different product, the pyrazoline (122), formed when the unsaturated ester (87) undergoes a 1,3-dipolar

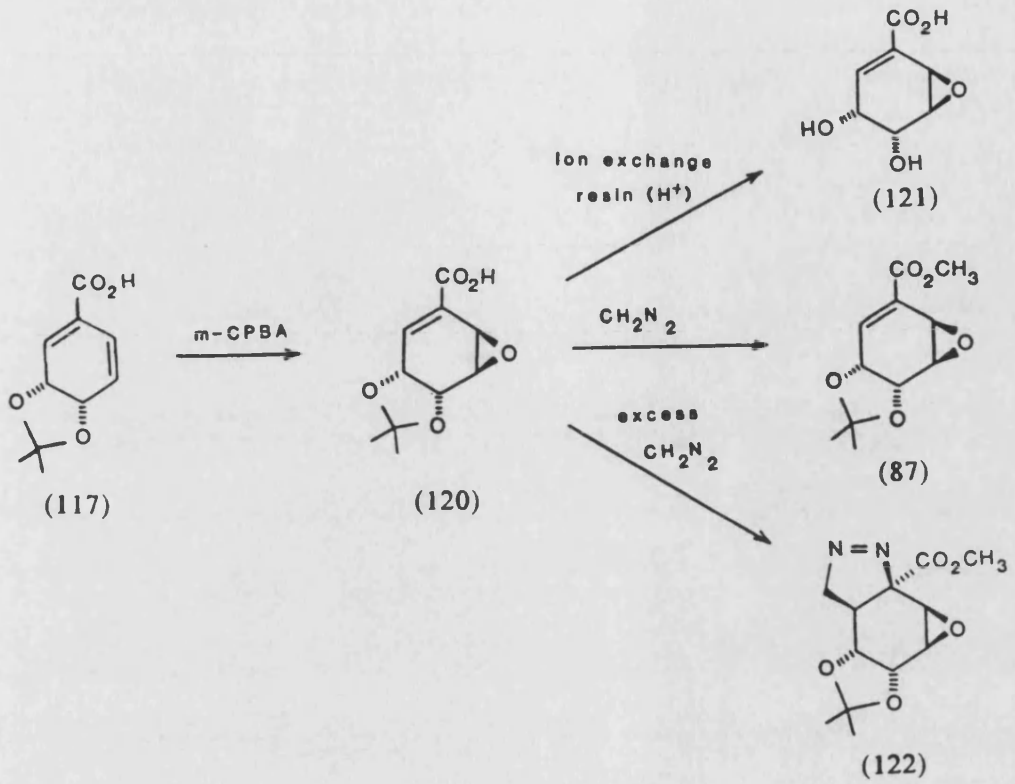
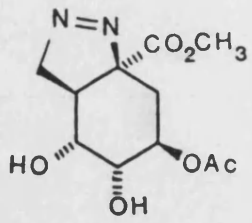


Fig. 2-25

cycloaddition with diazomethane[104].

The stereostructure (122) was deduced from ^1H n.m.r. coupling constants and NOEDS. The epoxide was known to have the β stereochemistry (relative to the isopropylidenedioxy group) and so it was only the orientation of the pyrazoline ring which required elucidation. Observed n.O.e. enhancements between the 7-*endo* proton and the 5-proton put these in close proximity, and an enhancement between one of the isopropylidene methyl groups and the 6-proton can only be reconciled with the structure indicated. Supporting evidence rests with the $J_{5,6}$ value of 3.0Hz - consistant with the di-equatorial or axial-pseudo equatorial arrangements which occur in the two possible conformers when the molecule has this configuration.

This relative stereochemistry follows the precedent of shikimic acid, such that diazomethane reacts from the most accessible face to give the β-pyrzoline (123)[105].



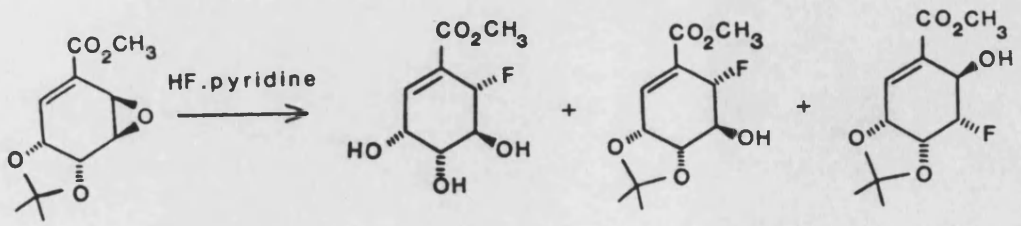
(123)

G. Fluorination of the Epoxide (87)

Having synthesised the epoxide (87) it was considered that the substituent most likely to realise the requirements outlined in the objectives would be a fluorine atom. A fluorine atom can replace a hydrogen atom without notable steric consequences due to the similarity in their Van der Waals radii ($r_F \approx 1.35$Å, $r_H \approx 1.10$Å), but may exhibit profoundly dissimilar chemical behaviour. These properties enable many fluorinated compounds to act as antimetabolites[106].

Consideration of some of the many reviews[107] dealing with epoxide cleavages led to the prediction that attack by the nucleophile would occur regioselectively at the 6 position.

Fluorination was accomplished using Olah's Reagent[108] (HF-pyridine 70% w/w) to furnish the methyl fluoroshikimate (124) as a colourless oil, in 49% yield (Fig. 2-26).



(87) (124) (125) (126)

Fig. 2-26

The fluorohydrins (125) and (126), with the protecting groups still in place were also obtained together in 3% yield (in a 4:1 ratio) and have been satisfactorily assigned on the basis of ^1H n.m.r.

The 400 MHz ^1H n.m.r. spectrum of methyl fluoroshikimate (124) in deuteriochloroform (Fig. 2-27) shows the 6-proton signal as a doublet of doublets centred at δ 5.39 exibiting the characteristic, large H-F geminal coupling ($J_{6,F}$ = 48.0Hz) and further coupling to 5-H ($J_{6,5}$ = 6.0Hz). Also, concealed, is a small allylic coupling (≈1Hz) to 2-H.

The resonance for 5-H appears as a double doublet of doublets, centred at δ4.38, with coupling constants $J_{5,F}$ = 17.0, $J_{5,4}$ = 9.0 and $J_{5,6}$ = 6.0 Hz. Comparing ^1H n.m.r. data[109] for vicinal H-F couplings in fluoro sugars it would be expected that $J_{H,F}$≥20Hz if both H and F atoms are axial, and $J_{H,F}$≤10Hz if both are equatorial. Since the observed value is intermediate between the two it can be concluded that either the 5-H or 6-F is *pseudo*- axial, but not both. The preferred chair conformation which best fits the observed couplings is that shown in Fig. 2-28, where the fluorine atom is equatorial and 5-H is axial.

Fig. 2-28

Evidently this conformer is held quite rigidly by intramolecular hydrogen bonding[110], since coupling constants observed in the more polar solvent d^4 methanol (Table 2-7) differ somewhat from those in $CDCl_3$; this perhaps indicates that a more

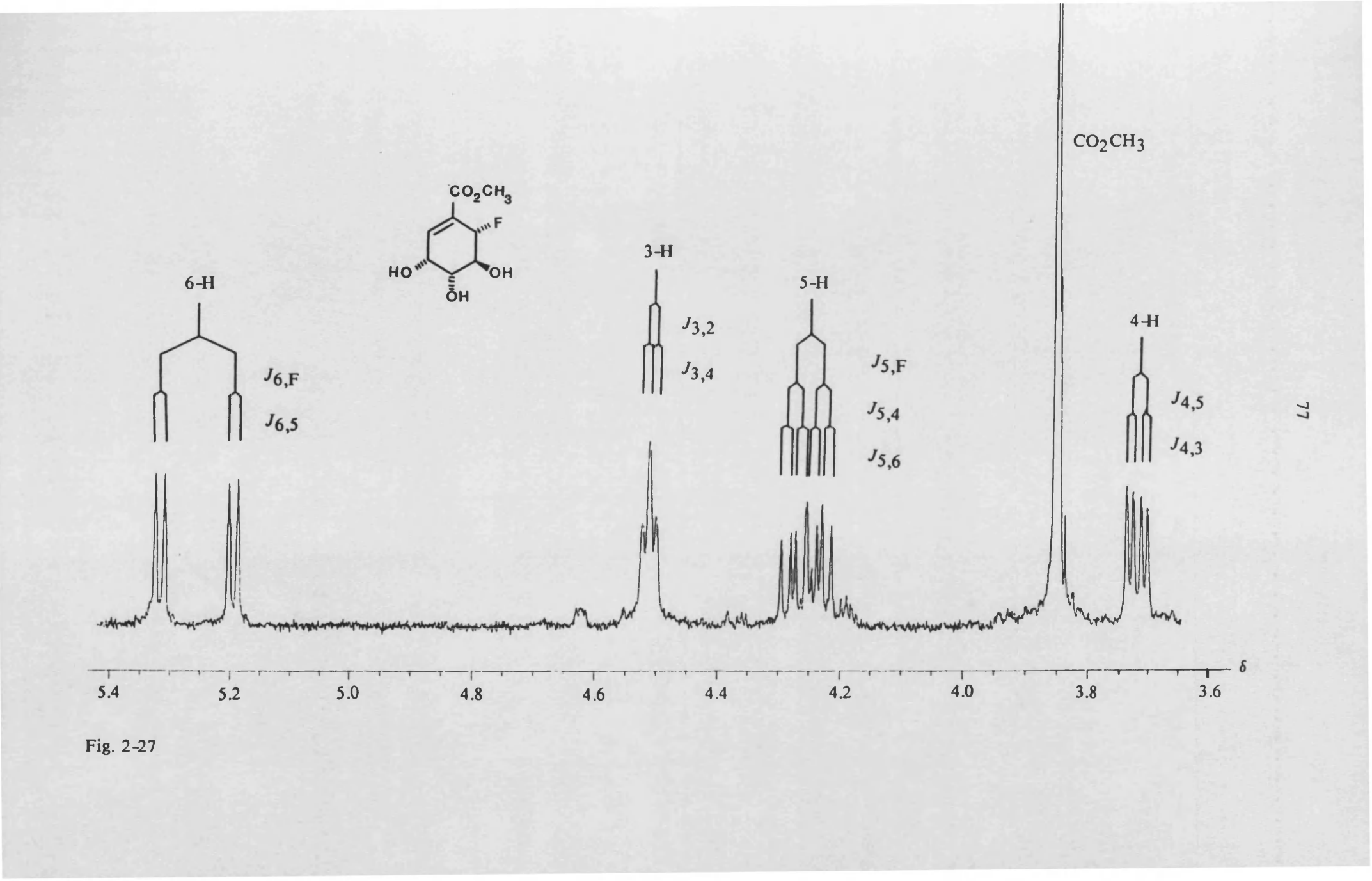

CO2CH3
F
HO
OH
OH
6-H
3-H
5-H
4-H
CO2CH3
J6,F
J6,5
J3,2
J3,4
J5,F
J5,4
J5,6
J4,5
J4,3
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
δ

Fig. 2-27

flexible or alternative conformation is adopted in methanol.

Table 2-7

SOLVENT	^{1}H N.M.R. COUPLING CONSTANTS (Hz)					
	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,F}$	$J_{6,F}$
$CDCl_3$	5.2	4.0	9.2	6.1	16.4	48.2
CD_3OD	4.0	7.1	8.2	5.1	17.2	47.6

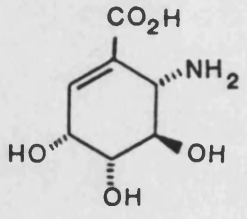
The ^{13}C n.m.r. spectrum shows ^{19}F-^{13}C coupling to all ring carbon atoms. The ^1J coupling ($J_{6,F}$=173 Hz),^2J couplings ($J_{1,F}$=18.7, $J_{5,F}$=21.2 Hz) and ^3J couplings ($J_{2,F}$=5.5, $J_{4,F}$=7.7 Hz) are all typical values[109,111]. ^4J coupling is unusual in aliphatic systems but is commonplace in aromatic systems and the observed splitting of $J_{3,F}$ = 2.0 Hz probably arises through homoallylic coupling.

1. Biological Activity

In the biological tests performed (Appendix IV) it was shown that the methyl fluoro-shikimate (124) exhibits a mildly inhibitory effect on the enzyme ESPS synthase. In this manner it mimics glyphosate, a powerful herbicide.

H. An Approach to Amination of the Epoxide (87)

The amine functionality is a well known surrogate alcohol, and as such has considerable potential as an inhibitor of the shikimic acid pathway. Although the amine (127) is not isosteric with shikimic acid it may bear close enough resemblance to (1) to act as a competitive inhibitor towards one or more of the relevent enzymes. .



(127)

Gassman *et al*[112] have described a method by which the ambident reactivity of the cyanide ion in nucleophilic ring opening of epoxides can be reversed; so that the use of trimethylsilyl cyanide (TMSCN) in the presence of zinc iodide affords the trimethylsilyl ether of a β-hydroxy-isonitrile. This is, in contrast to the trimethylsilyl ether of a β-hydroxy-nitrile produced by cataysis with aluminium chloride[113] (Fig. 2-29).

This reaction was carried out on the epoxy ester (87) to yield what appears to be

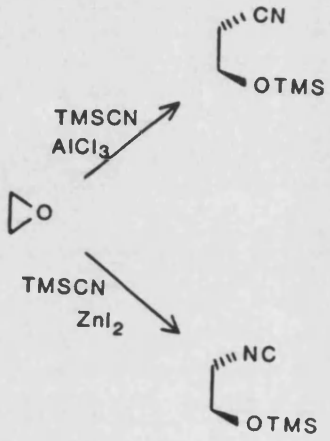
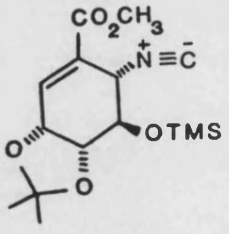


Fig. 2-29

the isonitrile (128).



(128)

Infrared spectroscopy is of no value in differentiating between a nitrile and isonitrile since neither a CN stretching band (expected[114] 2260-2240cm^{-1}) nor an NC

stretching band (expected[114] 2145-2135cm^{-1}) is observed.

The ^{1}H n.m.r. data suggests this compound to be the most likely despite the lack of corroborating evidence. Further analysis is necessary before the structure can be quoted with complete certainty, but shortage of the product and epoxide (87) prevented this. However, the chemical shift values suggest that the substituent is an isonitrile. This assumption is supported by the protons α and β to this group coupling with the nitrogen ($J_{N,6\text{-}H}$ = 2.0, $J_{N,5\text{-}H}$ = 2.5Hz). In contrast to nitriles the quadrupole relaxation of the nitrogen nucleus in isonitriles is so small that the ^{14}N - ^{1}H coupling becomes observable.

With the other coupling constants in this molecule all reasonable the structure of (128) appears to be entirely reasonable.

I. Summary

Various approaches to the synthesis of the epoxide (87) have been described, which produced some compounds with intriguing stereochemistry. The structures of all these substances have been rigorously and irrefutably determined.

A successful scheme was described whereby the epoxide was obtained in a regio- and stereoselective form. This compound allowed access to 6-substituted analogues of shikimic acid, and this was demonstrated by the synthesis of methyl fluoroshikimate. This compound showed minimal biological activity.

Reference is made to enzymic methods of ester hydrolysis - a method by which the resolution of enantiomers is also possible. This is the suggested method by which enantiospecific analogues of shikimic acid may be synthesised.

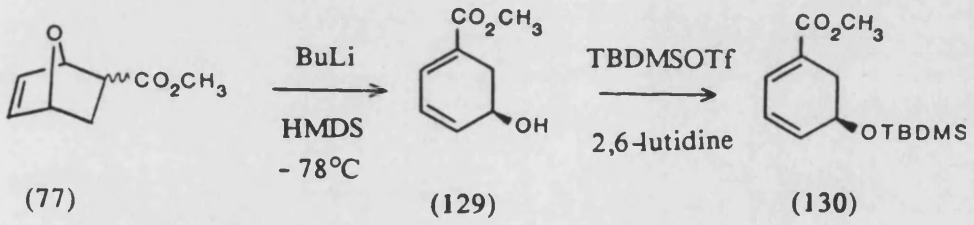
CHAPTER 3 : APPROACHES TO 3-, 4-, AND 5- SUBSTITUTED ANALOGUES OF SHIKIMIC ACID

A. 3- and 4- Substituted Analogues

A strategy has been formulated by which analogues of 3-*epi*- or 4-*epi*- shikimic acid can be obtained. This is described below.

1. Ring Opening and Protection of the Adducts (77)

The hydroxy diene (**129**) and *t*-butyldimethylsilyl ether (**130**) are both known compounds[19], which were obtained from the adducts (77) by a sequence of reactions shown below (Fig. 3-1).



(77) (129) (130)

Fig. 3-1

Base mediated ring opening of the adduct (77) with lithium hexamethyldisilazide by the procedure described earlier (see p.32) gave the hydroxydiene (**129**) as a colourless oil in 89% yield. Protection of the hydroxyl group as a TBDMS[115] ether, served both to reduce the air/moisture sensitivity of the compound and to influence subsequent reactions by virtue of its steric requirements. Protection was accomplished by using the highly reactive TBDMS triflate[116] and 2,6- lutidine at 0°C, affording the TBDMS ether (**130**), as an amber-coloured oil, in 81% yield. This product could be reacted without the need for purification.

2. Epoxidation Reactions

Treatment of the alcohol (129) with *m*-CPBA furnished a 4:1 mixture of *cis*- and *trans*- epoxy alcohols, (131) and (132), (Fig. 3-2) in 63% yield,

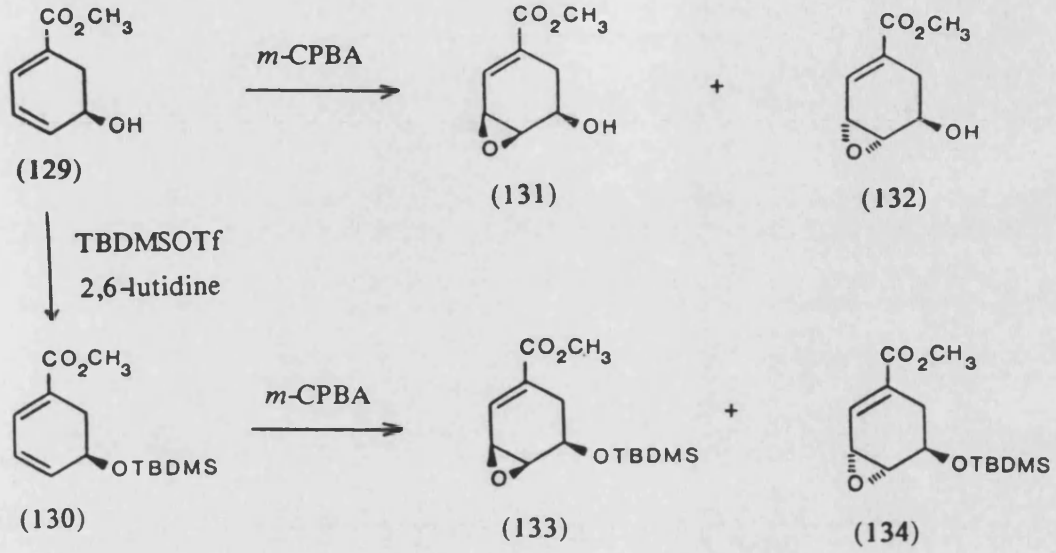


Fig. 3-2

whereas *m*-CPBA epoxidation of the silyl ether at 40°C afforded a 23:4 mixture of *trans*- and *cis*- epoxides (134) and (133) respectively. Epoxidation at ambient temperature gave almost entirely the *trans* isomer.

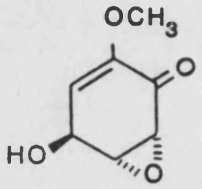
Clearly the orientation of epoxidation in the case of the diene (129) has been influenced by the free hydroxyl group, in a classic example of reagent approach control[78] so that the *cis* isomer predominates.

The proportion of the *cis* epoxide in the mixture is not as high as in other reported cases of epoxidation of allylic alcohols by peracids,[117] but McGowan and Berchtold[118] have found that this same reaction carried out at 0°C gives the isomers (131) and (132) in a ratio of 19:1.

Conversely, the sterically demanding TBDMS group dictates the direction of attack by the peracid on the protected compound (130), to give mainly the *trans*-epoxide (134).

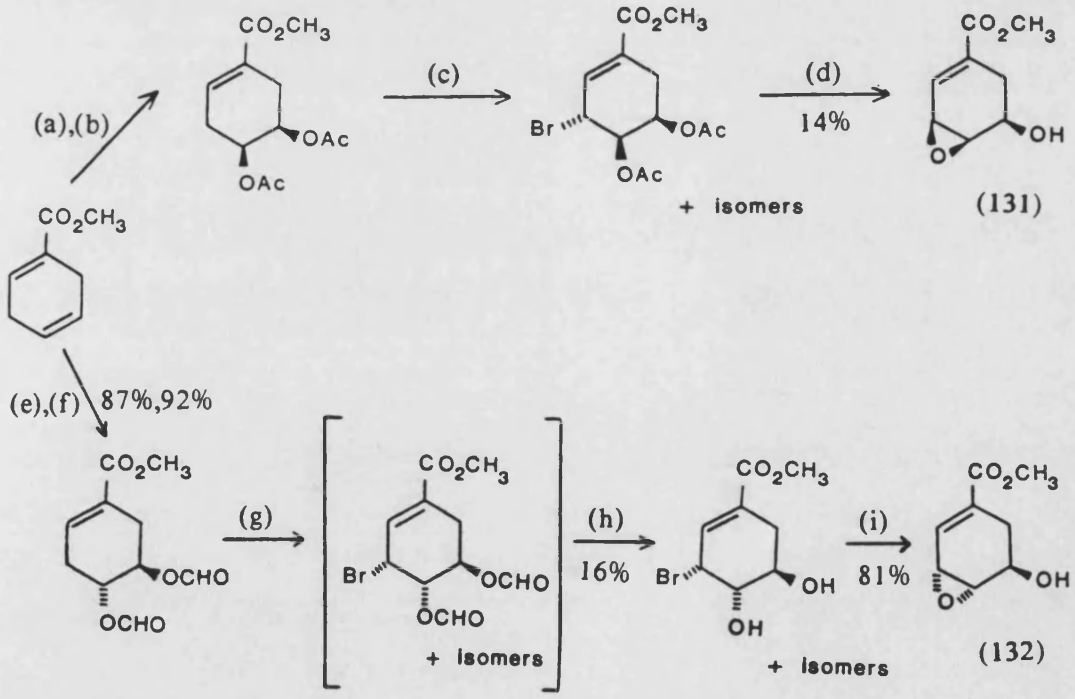
3. Literature Precedents

Compound (132) was first reported by Wickberg *et al.*[119] in a study of fungal metabolites antagonistic against *Fomes annosus* (root rot fungus). It was thought that the fungus *Chalara microspora* produced (132), and another compound, (135), which apparently showed activity. However, when prepared synthetically both proved inactive, and it was concluded that the observed activity was due to trace amounts of exceedingly active "impurities".



(135)

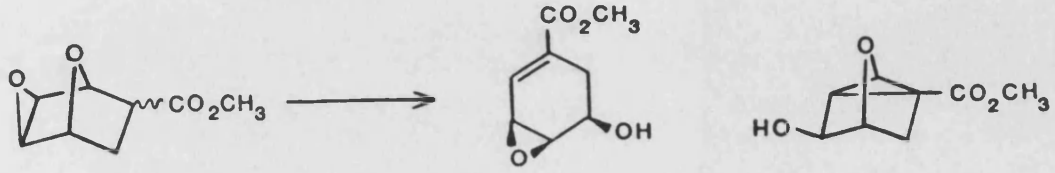
This group also synthesised the *cis*-isomer, and details of these syntheses are given in Fig. 3-3.



(a) I_2, AgOAc, AcOH; H_2O; (b) Ac_2O, $HClO_4$; (c) NBS, hν, CCl_4; (d) NaOMe, MeOH; (e) H_2O_2, HCO_2H; H_2O; (f) HCO_2H, P_2O_5; (g)NBS, hν, CCl_4/CS_2 (1:1); (h) MeOH, 5% phosphate buffer (pH = 7.5); (i) Na_2CO_3, NaBr, Me_2CO, reflux

Fig. 3-3

The alcohol (131) has been synthesised in Berchtold's laboratory[118] as an intermediate in his synthesis of 4-*epi*-shikimic acid, and has also been reported by Rodrigo *et al.*[20] as being present with the cyclopropane (137) in a 1:1 mixture derived from the base induced ring opening of epoxide (136) (Fig. 3-4). The yield of this reaction using lithium di-isopropylamide was apparently 85%, although similar experiments within the Bath group[43b] yielded only the cyclopropane (137) (65%).



(136) (131) (137)

Fig. 3-4

Rodrigo fails to quote experimental details and spectral data so no further comparison is possible.

Rodrigo also reports the synthesis of epoxides (133) and (134) in the same communication, but again no comparison is possible due to his omission of the relevant data.

4. NMR Comparisons

Table 3-1 shows that the ^{1}H n.m.r. data for the *cis*- and *trans*-epoxy alcohols (131) and (132) yielded in our reaction is generally in good accord with those already published.

An interesting feature of the ^{1}H n.m.r. spectra of these epoxy alcohols and their *O*-silyl derivatives (see Appendix I) is the interchanging of chemical shifts corresponding to 6α-H and 6β-H in the *cis* and *trans* isomers. The 6α-H resonance is consistently found downfield from the 6β-H in the *cis* compounds, and the reverse is true for the *trans* compounds.

It is well documented that in the absence of any substituent induced shielding

Table 3-1 Comparison of ^1H n.m.r. data reported for (131) and (132)

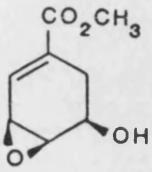
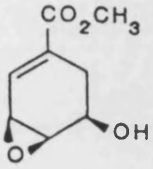
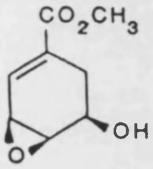
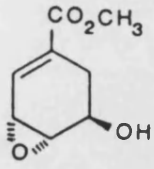
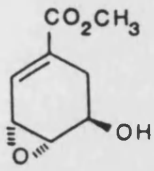
Compound	2-H	3-H	4-H	5-H	6α-H	6β-H	CO_2CH_3
	7.05	3.55	3.70	4.20	2.95	2.15	3.90
	7.02	3.53	3.67	4.15	2.92	2.13	3.76
	7.02	3.53	3.68	4.15	2.90	2.14	3.76
	7.25	3.50	3.55	4.65	2.40	2.90	3.85
	7.14	3.50	3.61	4.58	2.30	2.80	3.77

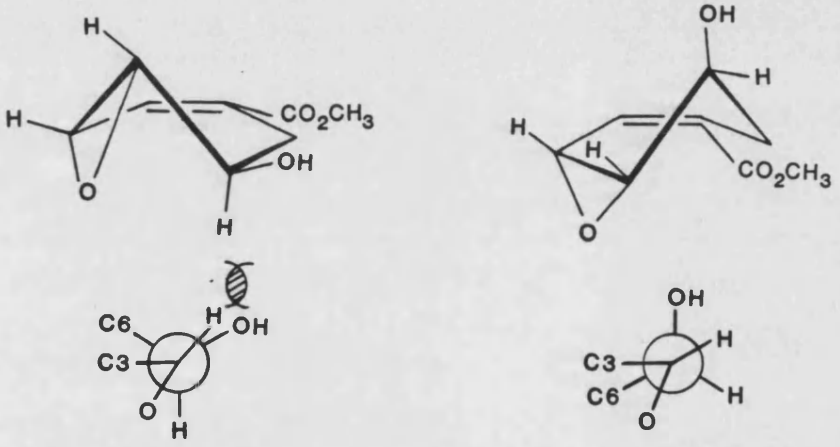
Table 3-1 contd.

$J_{2,3}$	$J_{2,6\alpha}$	$J_{2,6\beta}$	$J_{3,4}$	$J_{4,5}$	$J_{4,6\alpha}$	$J_{4,6\beta}$	$J_{5,6\alpha}$	$J_{5,6\beta}$	J_{gem}	REF
3.8		3.2	4.4	6.4	1.6		6.4	10.0	16.4	119
3.4			4.1							118
4.0		3.5	4.5	1.0	2.0		6.5	10.0	16.5	This work
4.1	3.0		4.0	2.5		1.8	5.0	1.8	17.0	119
4.0	3.5		3.5	2.0		2.0	5.0	2.5	17.5	This work

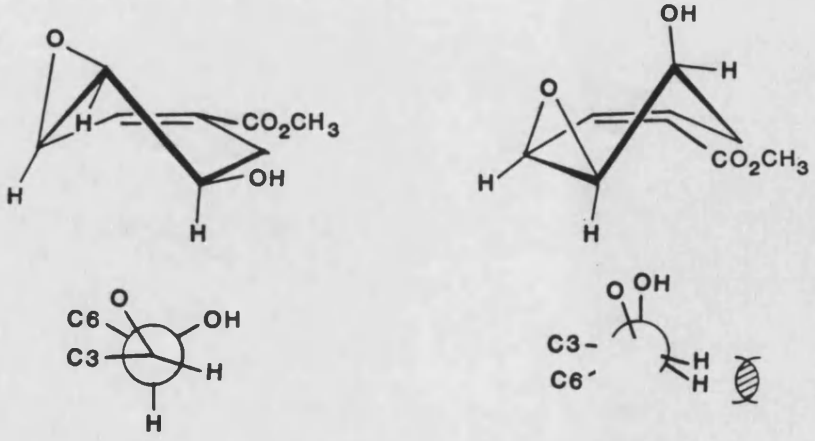
effects, an equatorial proton resonates at lower field than an axial proton attached to the same carbon atom[120]. There is little flexibility in the systems considered here, and the particular conformation adopted appears to owe less to any "preference" of a substituent to take up an equatorial orientation, than to the unfavourable gauche interactions which exist in the alternative conformer.

The relative merits of the alternative conformations (Fig. 3-5) will be discussed in terms of the epoxy alcohols (131) and (132), but the same reasoning applies for the *O*-silyl derivatives.

Fig. 3-5 shows the two possible conformations of the *trans*-epoxy alcohol (132). It can be seen from the Newman projection of the isomer (132a) that when the hydroxyl



(132a) (132b)



(131a) (131b)

Fig. 3-5

group is equatorial, an unfavourable gauche interaction arises between the 4-H and the 5-OH, due to these substituents being eclipsed. A similar manifestation occurs between the epoxidic oxygen and 5-H, but to a lesser extent since they are only partially eclipsed. The alternative conformer (132b) has the hydroxyl group axial which staggers the substituents and averts these unfavourable interactions. In this preferred situation the 6β proton is equatorial, and consequently it is this proton which resonates at lower field compared with the axial 6α proton.

In contrast, the unfavourable gauche interactions in the *cis*-isomer (131) arise when the hydroxyl is axial (131b), and so the preferred conformer has this group equatorial. In this conformation (131a) the 6α proton is equatorial, and hence is found at lower field.

5. Epoxide Opening Reactions

In seeking an entry into N-substituted analogues of 3- and 4-*epi*-shikimic acids, the epoxides (131) and (132) were reacted with trimethylsilyl azide ($TMSN_3$).

Treatment of the mixture with two equivalents of $TMSN_3$ in dichloromethane at ambient temperature merely resulted in silylation of the free hydroxyl groups (Fig. 3-6). A recent publication[121] however, credits this reagent with being an effective silylating agent, and not just a source of azide ions.

The resulting TMS ethers (138) and (139) were separated by flash chromatography. However, when the mixture (comprising ≈90% (131) and ≈10% (132)) was treated with the same reagent in the presence of two equivalents of zinc iodide at 40°C, a rapid reaction afforded the azide (140) in 59% yield. A combination of double resonance experiments, analysis of the ^1H n.m.r. coupling constants, and confidence in the relative stereochemistry of the starting material allowed us to deduce the stereostructure of the major reaction product.

For example, the coupling constant $J_{4,5}$ = 2.0Hz can only be reconciled with a *cis* arrangement of the 4- and 5- protons in (140), and since we know that the product is derived from the *cis*-epoxide, it follows that the only possible relative stereochemistry

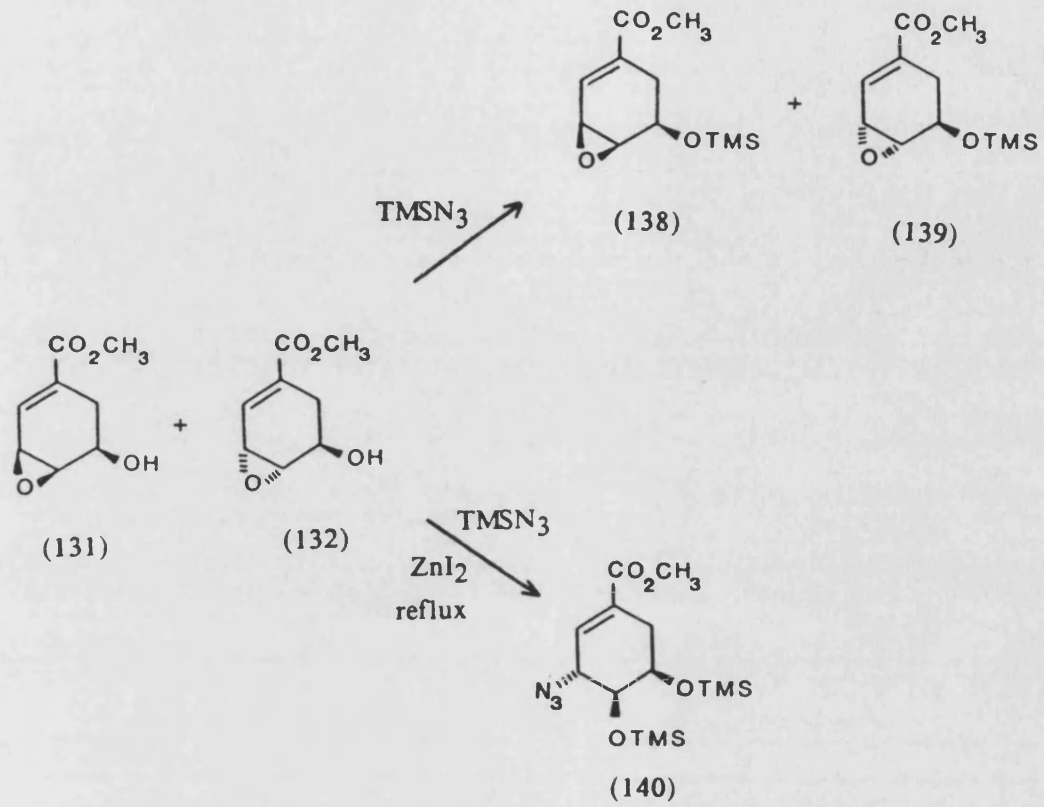


Fig. 3-6

is that shown.

Other products of the reaction were observed (5%) but due to the complexity of the ^1H n.m.r. spectrum they could not be identified individually. Three other isomers are possible and signals at δ6.61 and δ6.79 (corresponding to the 2-H resonances) are clearly visible, suggesting the existance of at least two of these.

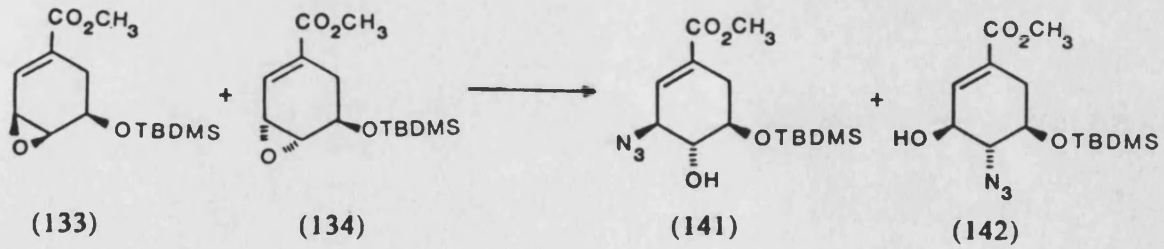


Fig. 3-7

In a similar experiment the 23:4 mixture of TBDMS ethers (133) and (134) was treated with $TMSN_3$ in dichloromethane at 40°C, but found to be inert. Upon the addition of zinc iodide, two products, were isolated (Fig. 3-7); (141) (56% from a theoretical maximum of 85%) and (142) (13% from a possible 15%).

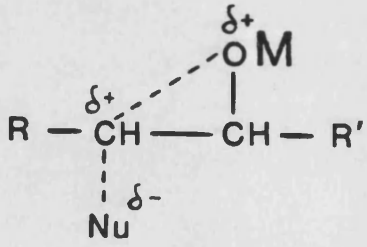
Once again, ^1H n.m.r. coupling constants allied with a sound knowledge of the starting materials led us to conclude the stereostructures of these compounds.

The $J_{4,5}$ coupling constant* (9.0Hz) for the azide (141) indicated a *trans* arrangement between these protons. Since (141) is the major reaction product it must have been derived from the *trans*-epoxide (134), and since 4-H was identified as the carbinol proton the stereostructure could be deduced.

For the minor product (142) the coupling constants $J_{3,4}$ = 8.5 and $J_{4,5}$ = 9.5, suggest a *trans* arrangement of these protons. In this case the 3-H resonance was identified as the carbinol proton and by a similar dialectic progression the relative stereochemistry was deduced.

(a) Rationalisation of Regioselectivity

At first glance the regiochemistry of the azides (140), (141) and (142) may appear capricious, but the position of nucleophilic attack in each case can be rationalised. Before this is possible we need to consider the mechanism by which these reactions take place.



(143)

* Resonances were assigned on the basis of decoupling experiments.

The opening of epoxides by nucleophiles[107] in basic or neutral media proceeds by an S_N2 mechanism at the most sterically accessible carbon. However, epoxide opening is facilitated by electrophilic assistance from protic solvents or Lewis acids, and under these conditions is thought to proceed through the transition state (143), where bond breaking has progressed to some extent (borderline A 2). Consequently there is a greater tendency for a nucleophile to attack at the carbon atom which can better accommodate a positive change in the transition state.

Taking these factors into consideration it would appear that the azide ion will attack preferentially at the 3-position of these epoxides, where a partial positive charge can be stabilised by resonance.

This is clearly the case for azide ion opening of (134) (illustrated in Fig. 3-8(i)) where C-3, C-4 and C-5 are shown schematically for clarity) although the TBDMS group would prevent attack at the 4-position in any case.

The 3-position is also the preferred site of attack for the epoxide (131) (Fig. 3-8(iii)), since there is only a very small amount of product formed *via* the mechanism (iii)b (less than 5%).

Epoxide (133), however, undergoes attack at the 4- position. Evidently attack at the 3- position is disfavoured since this would bring the oxygen-metal complex into unacceptably close proximity with the bulky TBDMS group, so the transition state (143) (Nu = N_3 , M = $^{-}ZnI_2$) is prevented from forming.

It is also interesting that silylation of the alcohol liberated from epoxide opening does not take place in those compounds which are already silylated. This, presumably, is as a consequence of the steric requirements of these groups.

6. Implications

It should therefore be possible, by careful choice of *cis*- or *trans*- epoxide, and selective utilisation of the TBDMS group, to obtain regioselective and stereoselective control of epoxide opening, to obtain all combinations of 3,4- amino alcohols.

Clearly the azides synthesised here are precursors to the amino *epi*-shikimic acids

(144), (145) and (146), after reduction of the azido group and suitable deprotections, but unfortunately a shortage of time prevented us from effecting successful azide

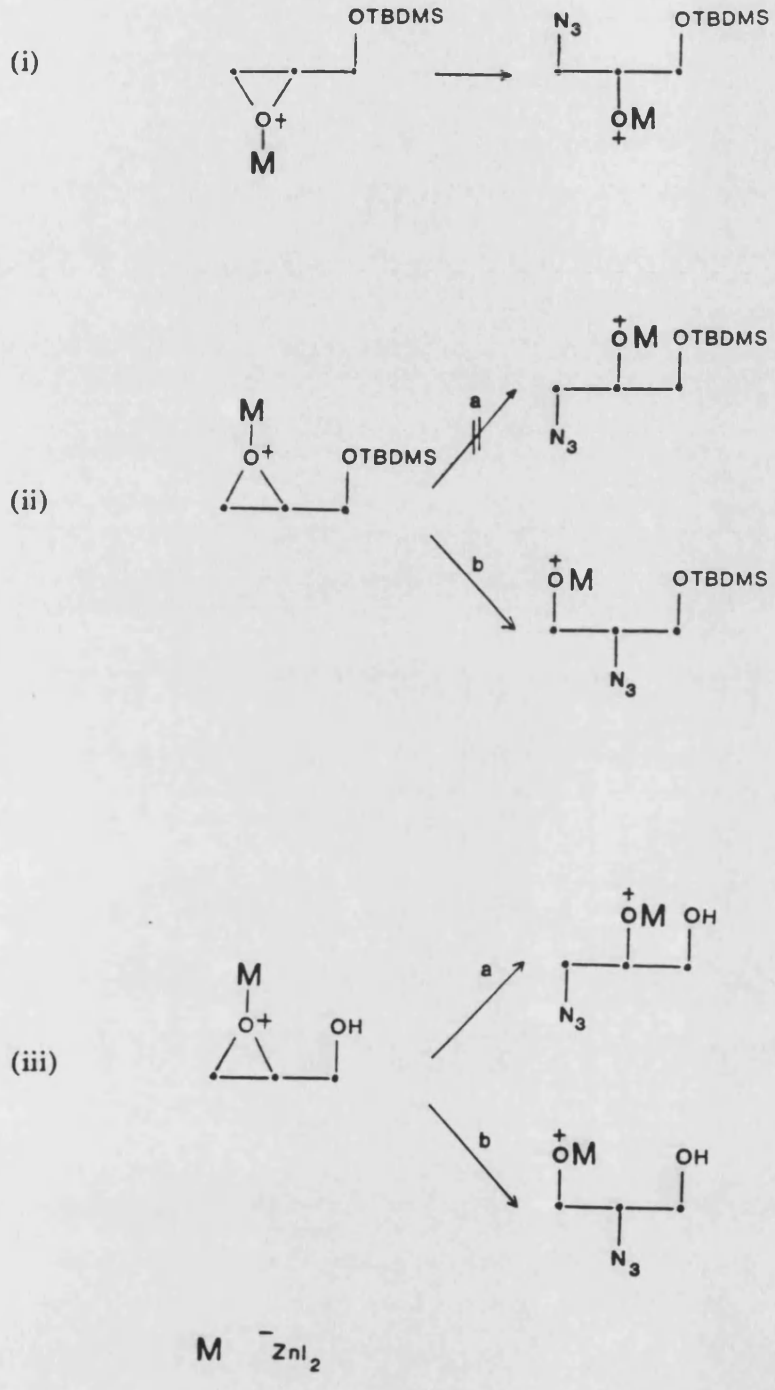
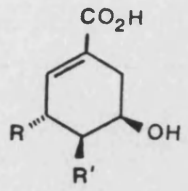
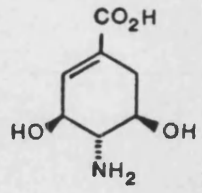


Fig. 3-8: Reaction of the epoxides (134), (133) and (131) with trimethylsilyl azide and zinc iodide. Schematic representation showing C-3, C-4 and C-5 of the substrate and products in profile (including relative stereochemistry).



(144) R = NH_2, R′ = OH
(145) R = OH, R′ = NH_2

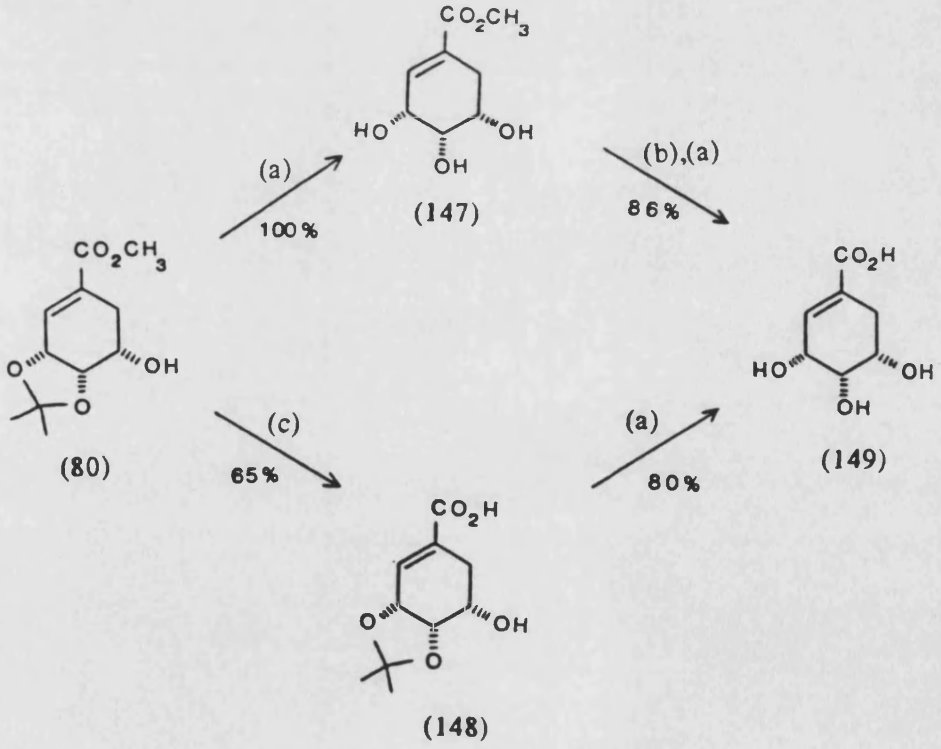


(146)

reduction, and completing the syntheses.

B. Synthesis of 5-*epi*-Shikimic Acid

A compound which bears a very close structural similarity to shikimic acid is 5-*epi*-shikimic acid (149). As such this analogue might be expected to exhibit some antagonistic effects against enzymes of the Shikimic Acid Pathway. (149) was obtained by simple deprotection of the hydroxy ester (80) (Fig. 3-9).



(a) Dowex 50W-X8 (H^+), MeOH; (b) KOH, dioxan; (c) PLE, H_2O-Me_2CO (9:1), phosphate butter (pH7); H^+

Fig. 3-9

Removal of the acetonide protecting group of (80) using strongly acidic ion exchange resin[103] was effected in quantitative yield. The resulting triol ester (147) was then subjected to base hydrolysis (KOH, dioxan) and subsequently acidified with ion exchange resin to furnish 5-*epi*-shikimic acid (149) in 86% yield (overall 86% from (80)).

An attempt was made to make the triol (149) in an optically active form by reversing the sequence of reactions and using an enzymic ester hydrolysis. However pig liver esterase showed no enantioselectivity and no kinetic resolution was obtained - the hydroxy acid (149) was furnished in 65% yield and was not optically active. The synthesis could be carried to completion by treating (148) with Dowex 50W-X8(H^+) ion exchange resin, to yield compound (149) in 80% yield (52% overall). Obviously a different choice of esterase may hold the key to making this product enantiospecifically.

Unfortunately 5-*epi*-shikimic acid exhibited no biological activity, but the compound did prove interesting in-as-much-as its ^1H n.m.r. spectrum when run in d^6 acetone, demonstrated two identical signals at δ6.65 and δ6.69, which together integrated to one proton. These clearly correlate with the 2-proton, and since no other signal exhibits this duplication, this suggests that rotameric forms of (148) have been resolved. The signals collapse to a single multiplet (at 6.66 p.p.m.) on addition of D_2O, and occur only as one multiplet when the spectrum is run using d^4 methanol as the solvent.

C Summary

Methods have been described by which 3-, and 4- substituted analogues of shikimic acid can by synthesised. By demonstrating this process with the azide ion, ground rules have been laid down whereby the regio- and stereoselectivity of the products arising from nucleophilic attack on the epoxide precursors can be predicted.

In addition, 5-*epi*-shikimic acid has been synthesised. The racemic form of this compound exhibits no biological activity, but a method has been suggested by which the synthesis may be made enantioselective.

EXPERIMENTAL

EXPERIMENTAL

General

Solvents and Reagents

Light petroleum refers to that fraction boiling in the range 60-80°C unless otherwise stated, and ether refers to diethyl ether. THF was distilled from a solution dried with sodium in the presence of benzophenone, and dichloromethane was purified by distillation from calcium hydride. Purification of reagents and other solvents followed the procedures outlined in *Purification of Laboratory Compounds*[122], with the exception of *m*-CPBA, which was purified by following the method of Fieser and Fieser[123].

Chromatography

T.l.c was performed on aluminium plates coated with kieselgel 60 F_{254} , and compounds were visualised by illumination with short wavelength (254nm) ultraviolet light, followed by treatment with one of the following : 7% (w/v) methanolic solution of phosphomolybdic acid; 0.5% (w/v) aqueous potassium permanganate solution; 5% (v/v) sulphuric acid in ethanol; iodine vapour. Sulphur compounds were visualised with 0.5% (w/v) aqueous palladium chloride solution containing a few drops of 25% (v/v) hydrochloric acid.

Flash chromatography was carried out using Merck 7747 or Merck 9385 silica gel.

Strectroscopy

N.m.r. spectra were recorded at 270MHz (^1H) or 67.8MHz (^{13}C) using deuteriochloroform ($CDCl_3$) as solvent unless otherwise stated. Typically the chloroform impurity contained in the $CDCl_3$ was used as an internal standard (δ7.27), but where necessary tetramethylsilane (TMS) was employed as an internal standard. The multiplicities of the resonances are denoted by s (singlet), d (doublet) - and multiples thereof, m (multiplet). The term br (broadened) is used where a signal is not

distinct, as in br m (broadened multiplet). I.r. spectra were recorded in $CHCl_3$ solutions unless otherwise stated. Mass spectra (m.s.) were recorded using the chemical ionisation (C.I.) technique (reagent gas isobutane) unless otherwise stated. All melting points (m.p.s.) are uncorrected. Microanalyses and X-ray crystallography were performed by the Physical and Chemical Measurement Unit (University of Bath).

Instrumentation

^1H n.m.r.	Jeol GX FT 400 (400 MHz)
	Bruker WM 400
	Jeol GX FT 270 (270MHz)
	Bruker AM 200 (200 MHz)
	Jeol PS 100 (100 MHz)
	Varian EM-360 (60 MHz)
	Hitachi Perkin-Elmer R24
^{13}C n.m.r.	Jeol GX FT 270 (68.7 MHz)
	Jeol FX 90Q (22.5 MHz)
I.r.	Perkin-Elmer 197
M.s.	VG 7070E with 2000 data system
	VG 70/250 SE
M.p.s.	Electrothermal MKII
	Kofler block
Optical rotation	Perkin-Elmer 241 polarimeter.

Kinetic Studies on Diene Acid (117) and Diene Ester (81) under Acidic and Basic Conditions - Experiments were carried out using stock solutions of 1mg cm^{-3} of ester (81) in acetonitrile, and 200mg of acid (117) in 70 cm^3 of water.

u.v. procedure : the acid or base solution ($3cm^3$) was run as a blank and then 30μL of stock solution squirted into the cell. The solution was shaken and returned to the spectrophotometer set for repeat scans (a variable time cycle was used). The reaction was followed to completion in some cases, but in others the end point was found either by re-running the sample which had been allowed to stand overnight, or by computer prediction of the end point. All data was processed by computer to obtain rate constant and half life values.

Typical h.p.l.c. procedure : a solution of $1cm^3$ of diene acid stock solution and $1cm^3$ of 1M NaOH were mixed. Aliquots of 100μL of the above were added to $1cm^3$ of water after time "t", and 10μL samples of this solution were injected onto the column.

2-methoxycarbonyl-7-oxabicyclo[2.2.1] hept-5-ene (77) - A dry flask was charged with furan ($16cm^3$, 0.22mol), methyl acrylate ($14cm^3$, 0.16mol), and zinc iodide (16g,0.05mol). The flask was flushed with nitrogen, sealed, and allowed to stir for 48h. at ambient temperature. The reaction mixture was then diluted with ethyl acetate (*ca.* $200cm^3$), and washed thoroughly with 0.1M sodium thiosulphate, and brine. The dried (Na_2SO_4) solution was concentrated under reduced pressure, and short path distillation of the residue afforded a mixture of the *endo*- and *exo*-esters (77) as a clear, colourless oil (7.7g, 32%) b.p. 78-80°C/0.4mmHg (Found : C, 62.3; H, 6.3. Calc. for $C_8H_{10}O_3$: C, 62,3; H, 6.5%); R_F0.72 and 0.67 [50% EtOAc - light petroleum]; ν_{max} 1720^{-1} (C=0). ^1H n.m.r. data are in accord with those of Nelson and Allen[46] : the *endo*-isomer (77a) has δ_H (100MHz) 1.55 (1H,dd,J_{gem} = 10.0, $J_{3endo,2}$ = 4.0Hz, 3endo-H), 2.09 (1H,ddd,J_{gem} = 10.0, $J_{3exo,2}$ = 8.0, $J_{3exo,4}$ = 4.0Hz, 3exo-H), 3.17 (1H,ddd,$J_{2,3exo}$ = 8.0, $J_{2,3endo}$ = 4.0, $J_{2,1}$ = 4.0Hz, 2-H), 3.63 (3H, s, OCH_3), 5.00 (1H, br d, 4-H), 5.16 (1H, br d, 1-H), 6.21 (1H, dd, $J_{5,6}$ = 6.0, $J_{5,4}$ = 2.0Hz,5-H),

6.43 (1H, dd, $J_{6,5}$ = 6.0, $J_{6,1}$ = 6-H); the *exo*-isomer (77b) has δ_H (100MHz) 1.57 (1H, dd, J_{gem} = 12.0, $J_{3endo,2}$ = 8.0Hz, 3endo-H), 2.16 (1H, ddd, J_{gem} = 12.0, $J_{3exo,2}$ = 4.0, $J_{3exo,4}$ = 4.0Hz, 3exo-H), 2.43 (1H, dd, $J_{2,3endo}$ = 8.0, $J_{2,3exo}$ = 4.0Hz, 2-H), 3.71 (3H, s, OCH_3), 5.06 (1H, br d, 4-H), 5.18 (1H, br s, 1-H), 6.37 (2H, br s, 5-, 6-H).

5,6-exo-cis-dihydroxy-2-methoxycarbonyl-7-oxabicyclo [2.2.1] heptane (78) - The adducts (77) (10.0g,64.9mmol) in THF ($35cm^3$) were treated with a 1% (w/v) solution of osmium tetroxide in *t*-butanol* ($10cm^3$,0.06mole%), and 30%aq. hydrogen peroxide ($8cm^3$, 70.5m mol), and the reaction stirred for 4 days at ambient temperature. After this time the solvents were evaporated to leave a green residue. Successive crystalisations with ethyl acetate afforded a mixture of the *endo*- and *exo*-methoxycarbonyl glycols (78) (7.0g,65%) (Found : C,51.2;H,6.4.Calc. for $C_8H_{12}O_5$: C,51.1;H,6.4%) R_F 0.55 and 0.45 [EtOAc]; ν_{max} 3370 (OH), 1730cm^{-1} (C=0). The *endo*-isomer (78a) has δ_H (100MHz) 1.60 (1H,dd,J_{gem} = 9.0, $J_{3endo,2}$ = 5.0Hz, 3endo-H), 2.10 (1H,ddd,J_{gem} = 9.0, $J_{3exo,2}$ = 8.0, $J_{3exo,4}$ = 4.0Hz, 3exo-H), 3.10 (1H,ddd,$J_{2,3exo}$ = 8.0, $J_{2,3endo}$ = 5.0, $J_{2,1}$ = 4.0Hz,2-H), 3.25 (2H,br s,2OH's), 3.68 (3H,s,OCH_3), 3.85 (2H,br s, 5-, 6-H), 4.32 (1H,d,$J_{4,3exo}$ = 4.0Hz, 4-H), 4.42 (1H,d,$J_{1,2}$ = 4.0Hz,1-H); δ_C 28.17(t,3-C), 43.61 (d,2-C), 51.79 (q,OCH_3), 70.91 and 73.73 (2d, 5-, 6-C), 82.24 and 82.78 (2d, 1-, 4-C), 171.95 (s, C=0); and the *exo*-isomer (78b) has δ_H (100 MHz) 1.58 (1H,dd,J_{gem} = 9.0, $J_{3endo,2}$ = 7.0Hz, 3endo-H), 2.10 (1H,br m, 3exo-H), 2.52 (1H,dd,$J_{2,3endo}$ = 7.0, $J_{2,3exo}$ = 4.0Hz,2-H), 3.30 (2H,br s,2OH's), 3.70 (3H,s,OCH_3). 3.85 (2H, br s, 5-, 6-H), 4.38 (1H,d,$J_{4,3exo}$ = 4.0Hz, 4-H), 4.54 (1H, br s,1-H); δ_C 28.82 (t,3-C), 42.69 (d,2-C), 51.90 (q,OCH_3), 73.24 and 73.35 (2d,5-,6-C), 81.53 and 84.08 (2d,1-,4-C), 173.25 (s,C=0).

*Prepared by the method of Daniels and Fischer[124].

5,6-cis-exo-isopropylidenedioxy-2-methoxycarbonyl-7-oxabicyclo[2.2.1]heptane (79) - The diols (78) (5.0g, 26.6mmol) were dissolved in acetone and stirred at 50°C with 2,2-dimethoxyropane ($60cm^3$, 4.90 mmol), *p*-toluenesulphonic acid monohydrate (0.2g,1.0mmol) and calcium chloride (10g). After 4h. the calcium chloride was filtered off and the filtrate concentrated under reduced pressure. The yellow residue was taken up in ethyl acetate (*ca.* $200cm^3$) and washed with a small volume of water (*ca.* $15cm^3$). The solution was dried (Na_2SO_4) and the solvent evaporated to yield a pale yellow oil which solidified on standing. Recrystallisation from ether-light petroleum afforded white crystals of the *endo*- and *exo*-carbomethoxy acetonides (6.0g, 99%) (Found : C,57.9; H, 5.3. Calc. for $C_{11}H_{16}O_5$: C,57.9; H,5.3%) R_F 0.68 and 0.55 [50% EtOAc - light petroleum]; ν_{max} $1725cm^{-1}$ (C=0). The *endo*-isomer (79a) has δ_H (100MHz) 1.26 and 1.37 (2x3H,2s,CMe_2), 1.70 (1H,dd,J_{gem} = 8.5,$J_{3endo,2}$ = 4.0Hz, 3endo-H), 1.86 (1H,ddd,J_{gem} = 8.5, $J_{3exo,2}$ = 7.5, $J_{3exo,4}$ = 4.0Hz, 3exo-H), 3.00 (1H, ddd, $J_{2,3exo}$ = 7.5, $J_{2,1}$ = 4.0, $J_{2,3endo}$ = 4.0Hz, 2-H), 3.72 (3H, s, OCH_3), 4.26 (2H, br s, 5-, 6-H), 4.43 (1H, d, $J_{4,3exo}$ = 4.0Hz, 4-H), 4.54 (1H, d, $J_{1,2}$ = 4.0Hz, 1-H); δ_C 24.97 and 25.79 (2q, $C(CH_3)_2$), 27.20 (t, 3-C), 43.01 (d, 2-C), 52.11 (q, OCH_3), 79.37 and 79.58 (2d, 5-, 6-C), 80.02 and 82.13 (2d, 1-, 4-C), 111.06 (s, $C(CH_3)_2$), 172.00 (s, C=0); and the *exo*-isomer (79b) has δ_H (100 MHz) 1.30 and 1.47 (2x3H, 2s, CMe_2), 1.58 (1H, dd, J_{gem} = 8.0, $J_{3endo,2}$ = 6.0Hz, 3endo-H), 2.11 (1H, ddd, J_{gem} = 8.0, $J_{3exo,2}$ = 4.0, $J_{3exo,4}$ = 4.0Hz, 3exo-H), 2.42 (1H, dd, $J_{2,3endo}$ = 6.0, $J_{2,3exo}$ = 4.0Hz, 2-H), 3.70 (3H, s, OCH_3), 4.24 (2H, br s, 5-, 6-H), 4.44 (1H, d, $J_{4,3exo}$ = 4.0Hz, 4-H), 4.66 (1H, br s, 1-H); δ_C 25.14 and 25.90 (2q, $C(CH_3)_2$), 27.85 (t, 3-C), 41.93 (d, 2-C), 52.28 (q, OCH_3), 78.66 and 81.26 (2d, 5-, 6-C), 81.91 and 82.24 (2d, 1-, 4-C), 111.76 (s, $C(CH_3)_2$), 173.03 (s, C=0).

Methyl (3α, 4α, 5α)-3,4-isopropylidenedioxy-5-hydroxy-cyclohex-1-ene-1-carboxylate (80) - A dry, 3-necked round bottomed flask, fitted with thermometer, pressure equalising dropping funnel and setum cap, was charged with 1,1,1,3,3,3-hexamethyldisilazane ($9.3cm^3$, 44.1 mmol) in anhydrous THF ($40cm^3$). This was stirred in an argon

atmosphere, and cooled in an acetone-CO_2 bath. *n*-Butyl-lithium (44.0 mmol) was added slowly *via* a syringe and after 35 mins. a solution of the acetonides (74) (10g, 43.0 mmol) in THF (100 cm^3) added dropwise so that the internal temperature did not rise above -72°C. The reaction was allowed to warm slowly and quenched at -15°C with saturated aqueous ammonium chloride. The product was extracted with dichloromethane, dried ($MgSO_4$) and concentrated to a yellow oil. This solidified on standing to yield the title compound as white crystals (8.89g, 89%) m.p. 77-79°C (from EtOAc - light petroleum) (Lit.,[19] 78-79°C); R_F 0.47 [50% EtOAc - petroleum ether]; ν_{max} 2400 (OH), 1705 (C=0), 1650 cm^{-1} (C=C); δ_H (400 MHz) 1.41 and 1.51 (2x3H, 2s, CMe_2), 1.98 (1H, d, $J_{OH,5}$ = 5.0Hz, OH), 2.48 (1H, m, J_{gem} = 19.0, $J_{6\alpha,5}$ = 10.5, $J_{6\alpha,3}$ = 2.5, $J_{6\alpha,2}$ = 2.5Hz, 6α-H), 2.65 (1H, bdd, J_{gem} = 10.5, $J_{6\beta,5}$ = 6.0Hz, 6β - H), 3.79 (3H, s, OCH_3), 3.95 (1H, br m, 5-H), 4.42 (1H, dd, $J_{4,3}$ = 6.5, $J_{4,5}$=3.0 Hz, 4-H), 4.74 (1H, ddd, $J_{3,4}$ = 6.5, $J_{3,2}$ = 3.5, $J_{3,6\alpha}$ = 2.5 Hz, 3-H), 6.78 (1H, ddd, $J_{2,3}$ = 3.5, $J_{2,6\alpha}$ = 2.5, $J_{2,6\beta}$ = 1.0 Hz, 2-H); δ_C 25.97 and 27.35 (2q, $C(CH_3)_2$), 27.57 (t, 6-C), 52.01 (q, OCH_3), 66.83 (d, 5-C), 72.91 and 75.41 (2d, 3-, 4-C), 109.92 (s, $C(CH_3)_2$), 129.07 (s, 1-C), 134.76 (d, 2-C), 166.67 (s, C=0); *m/z* 229 (MH^+, 19%), 197 (9), 139 (51). (Found : C, 60.0; H, 7.0. Calc. for $C_{11}H_{16}O_5$: C, 59.9; H, 7.0%).

Methyl (3α, 4α)-3,4-isopropylidenedioxy-cyclohexa-1,5-diene-1-carboxylate (81) - (*a*) A solution of the hydroxy ester (80) (2g, 8.77 mmol) and triphenylphoshine (2.5g, 9.53 mmol) in THF (15cm^3) was stirred at ambient temperature under nitrogen, and freshly distilled diethylazodicarboxylate* (1.5cm^3, 9.53mmol) was added slowly. After 2.5h. the solvent was evaporated, and the orange residue taken up in the minimum amount of toluene. The solution was allowed to stand overnight in a refridgerator, and

* Extreme caution must be exercised as DEAD is violently explosive upon heating. The reagent was distilled using a short path distillation apparatus under high vacuum, with an industrial heat gun. A safety screen is essential.

the resulting white crystals of *N,N*-diethoxycarbonyl hydrazine removed by filtration. The filtrate was applied to a column, and gradient elution with hexane to 10% ethyl acetate-hexane yielded the title compound as a white solid (0.92g, 50%) m.p. 54-57°C (Lit.,[19] 47-49°C); R_F 0.21 [EtOAc-hexane]. ν_{max} 1705cm^{-1} (C=0); δ_H 1.39 and 1.41 (2x3H, 2s, CMe_2), 3.80 (3H, s, OCH_3), 4.65 (1H, ddd, $J_{4,3}$ = 9.0, $J_{4,5}$ = 4.0, $J_{4,6}$ = 1.0 Hz, 4-H), 4.81 (1H, dd, $J_{3,4}$ = 9.0, $J_{3,2}$ = 4.0 Hz, 3-H), 6.04 (1H, ddd, $J_{5,6}$ = 10.0, $J_{5,4}$ = 4.0, $J_{5,2}$ = 1.0 Hz, 5-H), 6.54 (1H, br d, $J_{6,5}$ = 10.0 Hz, 6-H), 6.86 (1H, ddd, $J_{2,3}$ = 4.0, $J_{2,6}$ = 1.5, $J_{2,5}$ = 1.0 Hz, 2-H); δ_C 24.65 and 26.65 (2q, C $(CH_3)_2$), 52.06 (q, OCH_3) 69.61 and 70.70 (2d, 3-, 4-C), 105.53 (s, C $(CH_3)_2$), 122.27 (d, 5-C), 152.52 (d, 6-C), 126.99 (s, 1-C), 133.54 (d, 2-C), 165.67 (s, C=0); *m/z* 211 (MH^+, 22%), 153 (100); pK_a 4.03±0.09 (Found : C, 62.7; H, 6.9. Calc. for $C_{11}H_{14}O_4$: C, 62.9; H, 6.7%).

(*b*) The hydroxy ester (80) (100mg, 0.44 mmol) was dissolved in dry dichloromethane (1cm^3) and stirred at 0°C under ntrogen. A solution of the sulphurane (86) (400mg, 0.59 mmol) in dichloromethane (1cm^3) was added dropwise to it, and the reaction stirred for 2.5h. The solvent was evaporated and the resulting oil flash chromatographed (eluting with 20% EtOAc-light petroleum) to yield the diene ester (81) (37mg, 53% corrected yield) and starting material (80) (23mg) respectively.

(*c*) The hydroxy ester (80) (150mg, 0.66 mmol) was dissolved in dichloromethane (10cm^3) and stirred with pyridine (0.2 cm^3, 2.48 mmol) at 0°C. Trifluoromethane sulphonic anhydride (120μL, 0.71 mmol) was added to the solution dropwise, and after 1h. the ice bath was removed and the reaction heated to 40°C. After 7 days the reaction was allowed to cool, and washed thoroughly with saturated aqueous copper sulphate solution and brine, dried ($MgSO_4$) and evaporated to a brown oil. This was columned (1:10 EtOAc-light petroleum) to yield firstly a white solid (67mg, 28%) which slowly turned to a brown oil on prolonged exposure to air. This was identified as *methyl (3α, 4α, 5α)-3,4-isoropylidenedioxy-5-trifluoromethanesulphonyloxy-cyclohex-1-*

ene-1-carboxylate (85). The sample decomposed before its melting point. R_F 0.74 [50% EtOAc-petroleum ether]. ν_{max} : 1720 (C=0), 1655 (C=C), 1415 cm^{-1} (SO_2); δ_H(400MHz) 1.41 and 1.43 (2 x 3H, 2s, CMe_2), 2.48 (1H, br dd [partially obscured by 6α-H], $J_{6\beta,5}$ = 5.5Hz, 6β-H), 2.91 (1H, m [partially obscured by 6β-H], J_{gem} = 16.5, $J_{6\alpha,5}$ = 9.0, $J_{6\alpha,2}$ = 2.0, $J_{6\alpha,3}$ = 2.0 Hz, 6α-H), 3.80 (3H, s, OCH_3), 4.53 (1H, dd, $J_{4,3}$ = 5.5, $J_{4,5}$ = 2.5 Hz, 4-H), 4.83 (1H, ddd, $J_{3,4}$ = 5.5, $J_{3,2}$ = 3.5, $J_{3,6\alpha}$ = 2.0 Hz, 3-H), 5.11 (1H, ddd, $J_{5,6\alpha}$ = 9.0, $J_{5,6\beta}$ = 5.5, $J_{5,4}$ = 2.5 Hz, 5-H), 6.83 (1H, br m, 2-H); δ_C 25.27 (t, 6-C), 26.18 and 27.23 (2q, $C(CH_3)_2$), 52.43 (s, OCH_3), 73.14 and 73.26 (2d, 3-, 4-C), 82.81 (d, 5-C), 111.51 (s, CMe_2), 116.09 (s, CF_3), 127.18 (s, 1-C), 135.19 (d, 2-C), 165.61 (s, C=0); *m/z* 361 (MH^+, 77%), 153 (100). (**85**) was too unstable for accurate C and H analyses to be obtained.

Double Resonance Data for (85)

Signal Irradiated (Chemical Shift, δ)	Observed Resonance 2-H	5-H	3-H	4-H	6β-H	6α-H
Original Signal	br m	ddd	br m	dd	12 lines	br dd
2-H (6.83)	-	ddd	ddd	dd	ddd	dd
3-H (4.83)	better resolution	ddd	-	d	ddd	resolved into 8 lines
6-H (2.88)	br d	br s	dd	dd	-	-

Further elution gave a colourless oil (73mg) which was found by ^1H n.m.r. to comprise a mixture of the triflate (**78**) (15%, overall yield 43%) and the elimination product, the diene ester (**81**) (26%).

Methyl (3α, 4α, 5α,) 3,4-isopropylidenedioxy-5-(4-toluenesulphonyloxy)-cyclohex-1 ene-1-carboxylate (83) - The hydroxy ester (80) (100mg, 0.44 mmol) in CH_2Cl_2(2ml) was stirred with pyridine (150 μL, 1.85 mmol) at 0°C under N_2. *p*-Toluenesulphonyl choride (0.1g, 0.52 mmol) was added and stirring continued for 30 mins. The reaction was allowed to stand overnight in a refridgerator then poured into ice and extracted with ether. The etherial solution was washed thoroughly with sat $CuSO_4$ solution and brine, dried (Na_2SO_4) and the solvent evaporated to yield a white solid (61mg, 36%). m.p. 98-101°C (Lit.,[19] 102-103°C); ν_{max} 1720cm^{-1} (C=0); δ_H (60MHz) 1.34 (6H, s, CMe_2), 2.46 (3H, s, Ar-CH_3), 2.60 (2H, m, 6α-, 6β-H), 3.76 (3H, s,OCH_3), 4.40 (1H, m, 4-H), 4.74 (2H, m, 3-, 5-H) 6.72 (1H, br m, 2-H), 7.36 and 7.86 (4H, 2m, aromatic H's); *m/z* (E.I.) 367 (M-CH_3, 100%), 213 (74), 173 (41) (Found : C,56.7; H, 6.1. Calc. for $C_{18}H_{22}O_7S$: C, 56.5; H, 5.8 %).

Methyl (3α, 4α, 5α,) 3,4-isopropylidenedioxy-5-methanesulphonyloxy-cyclohex-1-ene-1-carboxylate (84) - To a solution of the hydroxy ester (80) (300mg, 1.42 mmol) in pyridine (5cm^3) at 0°C was added methanesulphonyl chloride (110 μL, 1.42 mmol) dropwise. Stirring was continued for 2h at 0°C, then for a further 1h as the reaction was allowed to warm to ambient temperature. The reaction was poured onto ice and extracted with ether. The etherial solution was washed with saturated copper sulphate solution, and brine, dried (Na_2SO_4) and evaporated under reduced pressure to give a white solid (320mg, 79%), m.p. 105-106°C (from ether-light petroleum) (Lit.,[19], 109-110°C); R_F 0.49 [50% EtOAc-light petroleum]; ν_{max} 1720cm^{-1} (C=0); δ_H 1.39 and 1.42 (2 x 3H, 2s, CMe_2), 2.80 (1H, m, J_{gem} = 16.5, $J_{6\alpha,5}$ = 10.0, $J_{6\alpha,2}$ = 2.5, $J_{6\alpha,3}$ = 2.5Hz, 6α-H), 2.87 (1H, br ddd, J_{gem} = 16.5, $J_{6\beta,5}$ = 5.5, $J_{6\beta,2}$ = 1.0, 6β-H), 3.13 (3H, s, SO_2CH_3), 3.79 (3H, s, OCH_3), 4.57 (1H, ddd, $J_{4,3}$ = 5.0, $J_{4,5}$ = 2.0, $J_{4,6\beta}$ = 1.0Hz, 4-H), 4.81 (1H, br m, 3-H), 4.94 (1H, ddd, $J_{5,6\alpha}$ = 10.0, $J_{5,6\beta}$ = 5.5, $J_{5,4}$ = 2.5 Hz, 5-H), 6.77 (1H, ddd, $J_{2,3}$ = 2.5, $J_{2,6\alpha}$ = 2.5, $J_{2,6\beta}$ = 1.0Hz, 2-H); δ_C 25.30 (t, 6-C), 26.49 and 27.57 (2q, C $(CH_3)_2$), 39.11 (q, SO_2CH_3), 52.22 (q, OCH_3), 73.46 and 73.84 (2d, 3-, 4-C), 75.30 (d, 5-C), 111.06 (s, C $(CH_3)_2$), 127.96 (s, 1-C), 135.22 (d, 2-C), 165.99 (s, C=0); *m/z* 307 (MH^+, 21%), 291 (14),

249 (100) (Found : C, 46.8; H, 5.8. Calc. for $C_{12}H_{18}O_7S$: C, 47.1; H, 5.9%).

Epoxidation of the Diene Ester (81). - (a) With m-chloroperbenzoic acid - A solution of the diene ester (**81**) (466mg, 2.22 mmol) in dichloromethane (15cm^3) was heated at 40°C with *m*-CPBA (480 mg, 2.77 mmol). After 12h. the reaction mixture was allowed to cool, and was washed with 10% aq. sodium sulphite, saturated sodium hydrogen carbonate solution, and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The resulting yellow oil was chromatographed with 10% ethyl acetate - light petroleum to yield a colourless oil, found by ^{1}H n.m.r. to be an 8:3 mixture of the epoxides (**87**) and (**88**). R_F = 0.65 [50% EtOAc - light petroleum]; ν_{max} 1720cm^{-1} (C=0). *Methyl (3α, 4α, 5β, 6β,) 5,6-isopropylidenedioxy-cyclohex-1-ene-1-carboxylate* (**87**) has δ_H (400 MHz) 1.38 and 1.39 (2 x 3H, 2s, CMe_2), 3.65 (1H, ddd, $J_{5,6}$ = 3.5, $J_{5,4}$ = 2.0, $J_{5,3}$ = 0.5 Hz, 5-H), 3.81 (3H, s, OCH_3), 3.98 (1H, ddd, $J_{6,5}$ = 3.5, $J_{6,2}$ = 1.5, $J_{6,4}$ = 0.5 Hz, 6-H), 4.56 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 2.5Hz, 3-H), 4.79 (1H, m, $J_{4,3}$ = 7.0, $J_{4,5}$ = 2.0, $J_{4,2}$ = 0.5, $J_{4,6}$ = 0.5Hz, 4-H), 6.81 (1H, ddd, $J_{2,3}$ = 2.5, $J_{2,6}$ = 1.5, $J_{2,4}$ = 0.5Hz, 2-H).

NOEDS Data for (**87**), from the epoxide mixture

Signal irradiated (Chemical shift, δ)	Observed n.O.e. (% enhancement)
5-H (3.65)	4-H (9), 6-H (10)
6-H (3.98)	5-H (7)
3-H (4.56)	2-H (9), 4-H (9)
4-H (4.79)	3-H (11), 5-H (7)

Methyl-1α, 2α,-epoxy- 3α,4α-isopropylidenedioxy-cyclohex-5-ene-1β-carboxylate (88) has

δ_H (400 MHz) 1.39 (6H, s, CMe_2), 3.80 (3H, s, OCH_3), 3.88 (1H, d, $J_{2,3}$ = 2.0Hz, 2-H), 4.47 (1H, ddd, $J_{4,3}$ = 7.0, $J_{4,5}$ = 2.5, $J_{4,6}$ = 1.5Hz, 4-H), 4.77 (1H, br dd. [partly obscured by 4-H of (87)], $J_{3,2}$ = 2.0Hz, 3-H), 5.86 (1H, ddd, $J_{5,6}$ = 10.5, $J_{5,4}$ = 2.5, $J_{5,3}$ = 0.5Hz, 5-H), 6.39 (1H, dd, $J_{6,5}$ = 10.0, $J_{6,4}$ = 1.5Hz, 6-H).

NOEDS Data for (88), from the epoxide mixture

Signal irradiated (Chemical shift, δ)	Observed n.O.e. (% enhancement)
2-H (3.88)	3-H (21)
4-H (4.47)	3-H (5), 2-H (4), OMe (37)

(b) With monoperphthalic acid - The diene ester (81) (100mg, 0.48 mmol) in ether ($3cm^3$) was treated with an etherial solution of monoperphthalic acid (0.49 mmol). After standing for 5 days at ambient temperature the reaction was washed with saturated sodium hydrogen carbonate and brine, dried (Na_2SO_4), and evaporated to a yellow oil. This was chromatographed (eluting with 1:10 EtOAc - light petroleum) to yield, initially, unreacted (81) (32mg). Further elution afforded a colourless oil comprising the epoxides (87) and (88) (25mg, 34% corrected yield) in a 3:1 ratio.

(c) With vanadium (IV) 2,4-pentadionate oxide - t-butyl hydroperoxide - A solution of the diene ester (81) (122mg, 0.58 mmol) and vanadium (IV) 2,4-pentadionate (0.4mg, 0.26 mol%) in benzene ($1cm^3$) was stirred at ambient temperature, under nitrogen. A solution of *t*-butyl hydroperoxide in toluene (3M, $0.48cm^3$, 1.44 mmol) was added, and after 12h. the solvents were evaporated to leave a golden-coloured oil. This was taken up in chloroform ($5cm^3$) and washed with 10% aq. sodium sulphite and brine, dried

($MgSO_4$) and concentrated under reduced pressure. The resulting oil was chromatographed with 1:10 ethyl acetate - light petroleum, to yield a 3:1 mixture of the epoxides (87) and (88) (60mg, 46%).

Methyl-1α, 2α,-epoxy- 3α,4α-isopropylidenedioxy-cyclohex-5-ene-1β-carboxylate (88) - The diene ester (81) (62mg, 0.30 mmol), potassium carbonate (0.07g) and benzonitrile (*ca.* 0.03 cm^3, 0.3 mmol) were stirred in methanol (5cm^3) at 0°C, and 30% aqueous hydrogen peroxide (*ca.* 0.04 cm^3, 0.35 mmol) was added dropwise. After 2.5h. 10% aqueous sodium sulphite (*ca.* 2 cm^3) was added and the organic material extracted with dichloromethane (x 3), dried ($MgSO_4$) and the solvents evaporated to leave a white semi-solid. This was chromatographed, eluting with 10% ethyl acetate - light petroleum, to furnish an oil which crystallised on standing to white needles of the title compound (67mg, quantitative) m.p. 60-71°C; R_F 0.79 [10% EtOAc - light petroleum]; ν_{max} 1720cm^{-1} (C=0); δ_H (400MHz) 1.38 (6H, s, CMe_2), 3.80 (3H, s, OCH_3), 3.88 (1H, d, $J_{2,3}$ = 2.0Hz, 2-H), 4.48 (1H, ddd, $J_{4,3}$ = 7.0, $J_{4,5}$ = 2.5, $J_{4,6}$ = 1.5Hz, 4-H), 4.77 (1H, br dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 2.0Hz, 3-H), 5.86 (1H, ddd, $J_{5,6}$ = 10.5, $J_{5,4}$ = 2.5, $J_{5,3}$ = 0.5Hz, 5-H), 6.40 (1H, dd, $J_{5,6}$ = 10.5, $J_{6,4}$ = 1.5Hz, 6-H); δ_C 25.75 and 27.49 (2q, $C(CH_3)_2$), 51.55 (s, 1-C), 52.78 (q, OCH_3), 54.71 (d, 2-C), 70.11 (d, 3-C), 70.55 (d, 4-C), 110.73 (s, $C(CH_3)_2$), 121.12 (d, 6-C), 131.72 (d, 5-C), 168.63 (C=0); *m/z* 227 (MH^+, 44%), 211 (9), 169 (100), 137 (34) (Found : C, 58.5; H,6.3. $C_{11}H_{14}O_5$ requires C, 58.4; H, 6.2%).

Methyl (3α, 4α, 5β, 6α,)-5-hydroxy-3,4-isopropylidenedioxy-6-phenylthio-cyclohex-1-ene-1-carboxylate (93) and Methyl (3α, 4α, 5α, 6β,)-5-hydroxy-3,4-isopropylidenedioxy-6-phenylthio-cyclohex-1-ene-1-carboxylate (94) - A 60% dispersion of sodium hydride in mineral oil (60mg) was washed with 40-60 petroleum ether and dried in a stream of N_2. THF (5cm^3) was added and the suspension cooled to 0°C. Thiophenol (0.15cm^3, 1.43 mmol) was added in one portion and the resulting white suspension stirred for 15 mins. before a solution of the three component epoxide mixture (87), (88) and (97)

(300mg, 1.45 mmol) in THF (5cm^3) was added. After 1.5h. the reaction was washed with 10% aqueous sodium hydroxide (x3). The aqueous phase was back extracted with dichloromethane and the organic portions combined and dried (Na_2SO_4). Concentration of this gave a yellow oil which was columned [1:2 ether - light petroleum (b.p. 30-40°C)] to yield (93) as a white solid (158mg, 35%)m.p. 126°C; R_F 0.56 [50% EtOAc-hexane]; ν_{max} 3680 - 3200 (OH), 1710cm^{-1} (C=0); δ_H (400MHz) 1.37 and 1.48 (2 x 3H), 2s, CMe_2), 2.45 (1H, d, $J_{5,OH}$ = 7.0 Hz, OH), 3.74 (3H, s, OCH_3), 3.90 (1H, ddd, $J_{5,4}$ = 9.0, $J_{5,OH}$ = 7.0 $J_{5,6}$ = 4.5Hz, 5-H), 4.29 (1H, dd, $J_{4,5}$ = 9.0, $J_{4,3}$ = 7.0Hz, 4-H), 4.40 (1H, d, $J_{6,5}$ = 4.5, 6-H), 4.60 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 3.5Hz, 3-H), 6.90 (1H, d, $J_{2,3}$ = 3.5 Hz, 2-H), 7.30 and 7.55 (5H, 2m, SPh); *m/z* (E.I.) 336 (M^+, 100%), 226 (38), 168(33) (Found : C, 60.7; H,6.0. $C_{17}H_{20}O_5S$ requires C,60.7; H, 5.6%).

Double Resonance Data for (93)

Signal irradiated (Chemical shift, δ)	Observed Resonance			
	3-H	6-H	4-H	5-H
Original resonance*	dd	d	dd	dd
2-H (6.90)	d	d	dd	dd
4-H (4.29)	d	d	-	d
5-H (3.90)	dd	s	dd	-

* After the addition of D_2O

Further elution with 2:3 ether - light petroleum afforded (94) (89mg, 20%) as a white solid m.p. 112°C; R_F = 0.47 [50% EtOAc-hexane]; ν_{max} (CH_2Cl_2) 3520 - 3600 (OH), 1710cm^{-1} (C = 0); δ_H (400MHz) 1.41 and 1.47 (2 x 3H), 2s, CMe_2), 2.26 (1H, br s, OH), 3.73 (3H, s, OCH_3), 4.02 (1H, dd, $J_{6,5}$ = 2.5, $J_{6,3}$ = 1.0Hz, 6-H), 4.37 (1H, dd, $J_{4,3}$ = 5.0, $J_{4,5}$ = 5.0Hz, 4-H), 4.58 (1H, br m, 5-H), 4.74 (1H, ddd, $J_{3,4}$ = 5.0, $J_{3,2}$ = 4.0, $J_{3,6}$ = 1.0Hz, 3-H), 6.78 (1H, d, $J_{2,3}$ = 4.0 Hz, 2-H), 7.29

and 7.60 (5H, 2m, SPh); *m/z* (E.I.) 336 (M^+, 336.1034. $C_{17}H_2O_5S$ requires 336.1029, 100%), 168 (38), 137 (49), 110 (100).

Double Resonance Data for (94)

Signal irradiated (Chemical shift, δ)	Observed Resonance			
	3-H	5-H	4-H	6-H
Original resonance*	ddd	dd	dd	dd
2-H (6.78)	br d	dd	dd	dd
6-H (4.02)	dd	d	dd	-

* After the addition of D_2O

NOEDS data for (94)

Signal irradiated (Chemical shift, δ)	Observed n.O.e. (% enchancement)				
	2-H	3-H	5-H	4-H	6-H
2-H (6.78)	-	6			
3-H (4.74)	8	-		6	
5-H (4.58)			-	9	7
4-H (4.37)		7	8	-	
6-H (4.02)			7		-

Methyl (3α, 4α, 5β, 6α,)-5-benzoyloxy-3,4-isopropylidenedioxy-6-phenylthio-cyclohex-1-ene-1-carboxylate (95) - A solution of the hydroxy sulphide (93) (33mg, 0.10mmol) in triethylamine (0.7cm^3) was cooled to 0°C and to the stirred solution was added benzoyl chloride (13μL, 1.1 mmol) in one portion. The reaction was allowed to warm to ambient temperature and was stirred overnight. Dichloromethane (*ca.* 5cm^3) was added and the solution washed with 10% H_2SO_4, saturated sodium hydrogen carbonate solution, and water, dried ($MgSO_4$) and concentrated under reduced pressure. The resulting oil was chromatographed with 20% ether - light petroleum (b.p. 30-40°C) to give the title compound as a colourless solid (34mg, 80%) m.p. 108-109°C; R_F 0.36 [1:2 Et_2O -light petroleum (b.p. 30-40°C)]; ν_{max} 1715 - 1700 cm^{-1} (2 C=0); δ_H (400MHz, C_6D_6) 1.20 and 1.40 (2 x 3H, 2s, CMe_2), 3.34 (3H, s, OCH_3), 4.42 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 3.5 Hz, 3-H), 5.00 (1H, dd, $J_{4,5}$ = 9.0, $J_{4,3}$ = 7.0Hz, 4-H), 5.18 (1H, d, $J_{6,5}$ = 4.0Hz, 6-H), 5.56 (1H, dd, $J_{5,4}$ = 9.0, $J_{5,6}$ = 4.0 Hz, 5-H), 6.96 (d, [obscured by Ph], 2-H), 6.68, 7.02, 7.56 and 7.89 (10H, 4m, SPh and OCOPh); *m/z* (E.I.) 440 (M^+, 440.1278. $C_{24}H_{24}O_6S$ requires M, 440.1291,2%) 331 (1), 202 (25), 105 (56), 91 (100)

NOEDS data for (95)

Signal irradiated (Chemical shift, δ*)	Observed Resonance (% enhancement)				
	2-H	5-H	6-H	4-H	3-H
5-H (5.56)		-	14	3	
6-H (5.23)		13	-	13	
4-H (5.00)		4	15	-	10
3-H (4.64)	13			16	-

* Measured in $CDCl_3$ - C_6D_6

Methyl (3α, 4α, 5α, 6β,)-5-benzoyloxy-3,4-isopropylidenedioxy-6-phenylthio-cyclohex-1-ene-1-carboxylate (96) - The hydroxy sulphide (94) (20mg, 0.06mmol) was dissolved in triethylamine (0.5cm^3) and the resulting solution stirred at 0°C. Benzoyl chloride (5.4 μL, 0.06mmol) was added in one portion and the reaction allowed to warm to ambient temperature and left overnight. Dichloromethane (*ca.* 5cm^3) was added, and the diluted reaction mixture washed with 10% H_2SO_4, saturated sodium hydrogen carbonate solution and water, dried ($MgSO_4$) and concentrated to a yellow oil. This was chromatographed with 20% ether - light petroleum (b.p. 30 - 40°C), to furnish the title comound as a white solid (17mg, 64%) m.p. 131-132°C; R_F 0.42 [1:2 Et_2O - light petroleum (b.p. 30-40°C)]; ν_{max} 1715 - 1700 cm^{-1} (2 C=0); δ_H (400MHz) 1.44 and 1.54 (2 x 3H, 2s, CMe_2), 3.63 (3H, s, OCH_3), 4.22 (1H, dd, $J_{6,5}$ = 2.5, $J_{6,3}$ = 1.0 Hz, 6-H), 4.53 (1H, br m, 4-H), 4.86 (1H, ddd, $J_{3,4}$ = 5.0, $J_{3,2}$ = 3.5, $J_{3,6}$ = 1.0Hz, 3-H), 6.11 (1H, dd, $J_{5,4}$ = 3.5, $J_{5,6}$ = 2.0 Hz, 5-H), 6.85 (1H, d, $J_{2,3}$ = 3.5 Hz, 2-H), 7.22-7.62 and 7.93 (10H, 2m, SPh and OCOPh); *m/z* (E.I.) 440 (M^+, 440.1311 $C_{24}H_{24}O_6S$ requires 440.1291, 2%) 331 (2), 105 (100).

Double Resonance Data for (96)

Signal irradiated (Chemical shift, δ)	Observed Resonance			
	5-H	3-H	4-H	6-H
Original resonance*	dd	ddd	br m	dd
2-H (6.87)	dd	br d	resolved into ddd	dd
5-H (6.11)	-	ddd	br d	s

Methyl (2α, 3α, 4α)-2-hydroxy-3,4-isopropylidenedioxy-cyclohex-6-ene-1-carboxylate (98)- Sodium hydride (7.6mg of 60% dispersion) was washed with light petroleum (b.p. 30-40°C) and dried in a stream of nitrogen. THF (0.5cm^3) was added and the stirred suspension cooled to 0°C. Thiophenol (10 μL, 0.19 mmol) was added and stirring continued for 20 mins. before a solution of epoxy ester (88) (40mg, 0.18 mmol) in THF (2.5cm^3) was added dropwise. After 1h. a few drops of water were added, and the solvents were evaporated under reduced pressure (the last traces of water removed under high vacuum) and flash chromatography of the resulting oil (gradient elution with 25% to 50% EtOAc - light petroleum) afforded the title compound as a colourless oil (9mg, 23%). R_F 0.34 [50% Et_2O-light petroleum]; ν_{max} 3620 - 2220 (CO_2H,OH) 1705 cm^{-1} (C=0); δ_H 1.35 and 1.36 (2 x 3H, 2s, CMe_2), 2.55 (1H, ddd, J_{gem} = 18.0, $J_{5\alpha,6}$ = 5.5, $J_{5\alpha,4}$ = 3.0 Hz, 5α-H), 2.66 (1H, ddd [partially obscured by OH], J_{gem} = 18.0, $J_{5\beta,4}$ = 5.0, $J_{5\beta,6}$ = 3.5Hz, 5β-H), 2.67 (1H, br s, OH), 3.79 (3H, s, OCH_3). 4.41 (1H, dd, $J_{3,4}$ = 6.5, $J_{3,2}$ = 2.5 Hz, 3-H), 4.54 (1H, ddd, $J_{4,3}$ = 6.5, $J_{4,5\beta}$ = 5.0, $J_{4,5\alpha}$ = 3.0 Hz, 4-H), 4.71 (1H, d, $J_{2,3}$ = 2.5 Hz, 2-H), 7.10 (1H, dd, $J_{6,5\alpha}$ = 5.5, $J_{6,5\beta}$ = 3.5 Hz, 6-H).

Methyl (3α, 4α)-3,4-dihydroxy-cyclohexa-1,5-diene-1-carboxylate (102) - The diene ester (81) (86mg, 0.41 mmol) was stirred at 56°C for 1.5h. with 50% aqueous acetic acid (4cm^3). The aqueous acid was partially removed on a rotary evaporator, and the product evaporated to a yellow oil under high vacuum. Trituration with ethyl acetate afforded the title compound as a white solid (36mg, 53%). The mother liquor was flash chromatographed with 50% EtOAc - light petroleum to give a further 23mg of (102) (33%, overall yield of 86%) m.p. 98.5°C (Lit.[19] 91-92°C);

R_F 0.15 [50% Et_2O - petroleum ether]; ν_{max} 3560 - 3140 (OH), 1715cm^{-1} (C=0); δ_H ($(CD_3)_2CO$) (3H, s, OCH_3), 3.80 (1H,br s, OH), 4.12 (1H,br s, OH), 4.15 [obscured by OH], 4-H), 4.40 (1H, dd, $J_{3,4}$ = 6.5, $J_{3,2}$ = 3.5 Hz, 3-H), 6.11 (1H, dd, $J_{5,6}$ = 10.0, $J_{5,4}$ = 5.0 Hz, 5-H), 6.39 (1H, br d, $J_{6,5}$ = 10.0 Hz, 6-H), 6.88(1H, br m, 2-H); *m/z* (E.I.) 170 (M^+, 23%) 152 (100), 138 (78) (Found : C, 56.5; H, 5.9. Calc.

for $C_8H_{10}O_4$: C, 56.5; H, 5.9%).

Methyl (3α, 4α, 5α, 6α,)-5,6-epoxy-3,4-isopropylidenedioxy-cyclohex-1-ene-1-carboxylate (103) and Methyl-3α,4α-5-dihydroxy-1α,2α,-epoxy-cyclohex-5-ene-1β-carboxylate (104) - A solution of the Dihydroxy diene ester (102) (28mg, 0.16 mmol) and *m*-CPBA (30mg, 0.16 mmol) in dichloromethane ($5cm^3$), was stirred at ambient temperature for 12h. Removal of the solvent under reduced pressure left a white gum which was chromatographed with 50% ethyl acetate - light petroleum, to furnish a 1:1 mixture of the title compounds (16mg, 52%) as a white semi solid. R_F 0.49 [EtOAc]; ν_{max} 3600 - 3180 (OH), 1720cm^{-1} (C=0); The 5,6-epoxide (103) has δ_H ($(CD_3)_2CO$ - D_2O) 3.74 (1H, br dd, $J_{5,6}$ = 2.5 $J_{5,4}$ = 1.5Hz, 5-H), 3.83 (3H, s, OCH_3), 4.00 (1H, dd, $J_{6,5}$ = 2.5, $J_{6,2}$ = 1.5Hz, 6-H), 4.09 (1H, br d, $J_{4,3}$ = 3.0 Hz, 4-H), 4.32 (1H, ddd, $J_{3,2}$ = 4.0, $J_{3,4}$ = 3.0, $J_{3,5}$ = 1.5 Hz, 3-H), 7.17 (1H, dd, $J_{2,3}$ = 4.0, $J_{2,6}$ = 1.5Hz, 2-H); and the 1,2-epoxide (104) has δ_H 3.81 (3H, s, OCH_3), 3.91 (1H, br d, $J_{2,3}$ = 1.5Hz, 2-H), 4.04 (1H, br d, $J_{3,4}$ = 3.0 Hz, 3-H), 4.18 (1H, ddd, $J_{4,5}$ = 3.5, $J_{4,3}$ = 3.0, $J_{4,2}$ = 1.0 Hz, 4-H), 6.27 (1H, dd, $J_{5,6}$ = 7.0, $J_{5,4}$ = 3.5Hz, 5-H), 6.48 (1H, d, $J_{6,5}$ = 7.0Hz, 6-H).

Reactions of the Hydroxy Ester (80) with Sodium Hydride-Thiophenol - (a) - A 60% dispersion of sodium hydride (40mg) was washed with light petroleum (b.p. 30-40°C) (x3) and dried in a stream of nitrogen. THF ($2cm^3$) was added and the suspension cooled to 0°C in an ice bath. Thiophenol ($0.1cm^3$, 1 mmol) was added in one portion and the white suspension stirred for 1h. before a solution of the hydroxy ester (80) (228mg, 1mmol) in THF ($3cm^3$) was added dropwise. The ice bath was removed and the reaction stirred at ambient temperature for 18h. after which time the solvent was evaporated, and the resulting brown oil applied to a silica column. Elution with 1:2 ethyl acetate - light petroleum gave *Methyl (1β,2β,3α,4α,5α)-5-hydroxy-3,4-isopropylidenedioxy-2-phenylthio-cyclohexane-1-carboxylate* (**108**) as a white solid (127mg, 50% corrected yield) m.p. 84.5-85°C (from ether-light petroleum); R_F 0.45 [50% EtOAc - light petroleum]; ν_{max} 3570(OH), 1720 cm^{-1} (C=0); δ_H (400MHz) 1.39 and 1.52 (2 x 3H, 2s, CMe_2), 1.95 (1H, ddd, J_{gem} = 14.0, $J_{6\alpha,5}$ = 9.0, $J_{6\alpha,1}$ = 6.5Hz, 6α-H), 2.17 (1H, ddd, J_{gem} = 14.0, $J_{6\beta,1}$ = 6.5, $J_{6\beta,5}$ = 6.0Hz, 6β-H), 2.27 (1H, d, $J_{OH,5}$ = 5.0 Hz, OH), 3.26 (1H, ddd, $J_{1,6\beta}$= 6.5, $J_{1,6\alpha}$= 6.5, $J_{1,2}$ = 4.5Hz, 1-H), 3.48 (1H, dd, $J_{2,3}$ = 6.0, $J_{2,1}$ = 4.5Hz, 2-H), 3.71 (3H, s, OCH_3), 4.15 (1H, br m, 5-H), 4.43 (1H, dd, $J_{4,3}$ = 6.0, $J_{4,5}$ = 3.5Hz, 4-H), 4.55 (1H, dd, $J_{3,2}$= 6.0, $J_{3,4}$ = 6.0Hz, 3-H), 7.24-7.34 and 7.47 (5H, 2m, SPh); δ_C 25.27 and 27.29 (2q, $C(CH_3)_2$), 28.61 (t, 6-C), 40.80 (d, 1-C), 50.36 (d, 2-C), 51.96 (q, OCH_3), 65.23 (d, 5-C), 75.38, and 76.92 (2d, 3-, 4-C), 109.34 (s, C $(CH_3)_2$), 127.65 (d, aromatic *p*-CH), 129.12 and 132.42 (2d, aromatic *o-m*-CH) 134.57 (s, aromatic C-S), 173.42 (s, C=0); *m/z* (E.I.) 338 (M^+, 100%), 171(78), 110(16). (Found : C 60.4; H, 6.6. $C_{17}H_{22}O_5S$ requires C, 60.4; H, 6.5%).

NOEDS data for (108)

Signal irradiated (Chemical shift, δ)	Observed n.O.e. (% enhancement)
2-H (3.48)	1-H(21), 3-H(5), aromatic *o*-H(5)
3-H (4.55)	2-H(5), 4-H(32)

Continued elution yielded an oil, found by ^1H n.m.r. to be a 1:1 mixture of starting material (80) and *Methyl (1α 2β 3α 4α 5α)-5-hydroxy-3,4-isopropylidenedioxy-2-phenylthio-cyclohexane-1-carboxylate* (109) (113mg, 23% corrected yield). The R_F 0.31 [50% EtOAc - petroleum ether] was coincidental with that of the hydroxy ester (80). (109) has δ_H (400MHz) 1.38 and 1.51 (2 x 3H, 2s, CMe_2), 2.00 (1H, d, $J_{OH,5}$ = 8.0Hz, OH), 2.46 (3H, m, 1-, 6α-, and 6β-H), 3.21 (1H, dd, $J_{2,1}$= 11.5, $J_{2,3}$ = 9.0Hz, 2-H), 3.69 (3H, s, OCH_3), 3.82 (1H, br m, 5-H), 4.01 (1H, dd, $J_{3,2}$= 9.0, $J_{3,4}$ = 5.0Hz, 3-H), 4.29 (1H, dd, $J_{4,3}$= 5.0, $J_{4,5}$ = 4.0Hz, 4-H), 7.28-7.33 and 7.56 (5H, m, SPh); δ_C 25.97 and 27.92 (2q, $C(CH_3)_2$), 32.00 (t, 6-C), 44.73 (d, 1-C), 50.29 (d, 2-C), 52.00 (q, OCH_3), 67.08 (d, 5-C), 76.04 and 77.37 (2d, 3-, 4-C), 109.75 (s, $C(CH_3)_2$), 128.31, 128.79 and 131.61 (3d, aromatic CH), 135.12 (s, aromatic C-s), 172.86 (s, C=0); *m/z* 339 (MH^+, 7%), 281(41), 171(100).

(b) A 60% dispersion of sodium hydride in mineral oil (180mg) was washed with pentane (x3) and dried in a stream of nitrogen. Anhydrous THF (5cm^3) was added and the suspension stirred at 0°C. Thiophenol (0.45cm^3, 4.4 mmol) was added and after 30 mins. a solution of the hydroxy ester (80) (1.0g, 4.4 mmol) in THF (5cm^3) was added slowly. After four days at ambient temperature a small amount of water (*ca.* 3cm^3) was added and the product extracted with chloroform. The solvent was dried ($MgSO_4$) and removed to leave a yellow oil which was flash chromatographed,

eluting with 1:2 EtOAc - light petroleum to yield initially *endo-3,4-isopropylidenedioxy-exo-2-phenylthio-6-oxabicyclo [3.2.1] octan-7-one* (112), as a cream-coloured solid (148mg, corrected yield 12%). m.p. 87-88°C (from ether-light petroleum); R_F 0.64 [50% EtOAc - light petroleum]; ν_{max} = 1760 (C=0), 1575cm^{-1} (C=C); δ_H 1.30 and 1.54 (2 x 3H, 2s, CMe_2), 2.30 (1H, br dd, J_{gem} = 13.0Hz, $J_{8endo,5}$ = 1.0 Hz, 8 *endo*-H), 2.38 (1H, ddd, J_{gem} = 13.0, $J_{8exo,5,1}$ = 5.5, J_{8exo} = 2.0Hz, 8 *exo*-H). 2.63 (1H, m, 1-H), 3.98 (1H, ddd, $J_{2,1}$ = 2.5, $J_{2,3}$ = 1.0, $J_{2,8endo}$ = 0.5Hz, 2-H), 4.21 (1H, dd, $J_{4,3}$ = 6.0, $J_{4,5}$ = 2.5Hz, 4-H), 4.40 (1H, br d, $J_{3,4}$ = 6.0Hz, 3-H), 4.67 (1H, ddd, $J_{5,8exo}$ = 5.5, $J_{5,4}$ = 2.5, $J_{5,8endo}$ = 1.0Hz, 5-H); δ_C 25.60 and 25.31 (2q, $C(CH_3)_2$), 28.74 (t, 8-C), 39.37 (d, 1-C), 44.94 (d, 2-C), 72.64 and 75.72 (2d, 3-, 4-C), 78.07 (d, 5-C), 109.84 (s, CMe_2), 128.2 (d, aromatic *p*-CH), 129.50 and 131.78 (2d, aromatic *o*-, *m*-CH), 132.13 (s, aromatic C-S), 175.41 (s, C=0); *m/z* (E.I.) 306 (M^+, 70%), 291 (100) (Found : C, 62.7; H, 6.0. $C_{16}H_{18}O_4S$ requires C, 62.7; H, 5.9 %). Continued elution afforded the following compounds (in order of elution) : *Methyl (1β,2β,3α,4α,5α)-5-hydroxy-3,4-isopropylidenedioxy-2-phenylthio-cyclohexane-1-carboxylate* (108) as a white solid (125mg, corrected yield 9%, m.p. 84.5-85°C), R_F 0.33. *Methyl (1α,2β,3α,4α,5α)-5-hydroxy-3,4-isopropylidenedioxy-2-phenylthio-cyclohexane-1-carboxylate* (109) (5% corrected yield) together with unreacted (80) in the proportions of 1:1, as an oil (125mg) R_F 0.24. ^{1}H n.m.r. data for (108) and (109) were identical to those of the characterised compounds obtained in (*a*).

Methyl (1β,2β,3α,4α,5α)-5-(4-bromobenzoyloxy)-3,4-dihydroxy-2-phenylthio-cyclohexane-1-carboxylate (110) - To a solution of pheynylthio hydroxy ester (108) (45mg, 0.13 mmol) and *p*-bromobenzoyl chloride (30mg, 0.14 mmol) in dichloromethane (3cm^3) under nitrogen was added freshly distilled triethylamine (19 μL, 0.13 mmol). After 5 days at ambient temperature the solvent was evaporated and the residue flash columned with 20% EtOAc-light petroleum to yield the *p*-bromobenzoate (110) as a white solid (19mg, 30%) m.p. 140-142°C; R_F 0.73 [50% EtOAc - light petroleum]; ν_{max} 3640-3280(OH), 1730-1720 cm^{-1} (C=0); δ_H 2.05 (2H,br s, 2OH) 2.15 (1H, ddd, J_{gem} =

14.0, $J_{6\beta,1}$ = 8.0, $J_{6\beta,5}$ = 3.5Hz, 6β-H), 2.33 (1H, ddd, J_{gem} = 14.0, $J_{6\alpha,5}$ = 7.0, $J_{6\alpha,1}$ = 4.5Hz, 6α-H), 3.44 (1H, br m, 1-H), 3.71 (4H, br s, OCH_3, 2-H), 4.28 (1H, dd, $J_{3,2}$ = 6.0, $J_{3,4}$ = 3.0Hz, 3-H), 4.40 (1H, dd, $J_{4,5}$ = 3.5, $J_{4,3}$ = 3.0Hz, 4-H), 5.44 (1H, ddd, $J_{5,6\alpha}$ = 7.0, $J_{5,4}$ = 3.5, $J_{5,6\alpha}$ = 3.5Hz, 5-H), 7.25-7.60 and 7.92 (9H, 2m, SPh and $OCOC_6H_4Br$); *m/z* 465 and 463 (MH^+-18, both 2%), 385(1), 383 (1), 294 (4), 279 (5), 203 (100), 202 (100), 201 (54), 200 (54).

Reaction of the Diene Ester (81) with Sodium Hydride-Thiophenol - Sodium hydride (30mg of a 60% dispersion) was washed with pentane (3x2cm^3) and dried in a stream of nitrogen. THF (2cm^3) was added and the suspension cooled in an ice-salt bath. Thiophenol (*ca.* 0.1cm^3) was added, and after 30 mins. a solution of the diene ester (81) (213mg, 1.01 mmol) in THF (2cm^3) was added dropwise. This was stirred for 1h. after which time a few drops of water were added, and the reaction mixture diluted with diethyl ether and dried ($MgSO_4$). The solvents were evaporated and the residual brown oil chromatographed with 20% ether-light petroleum (b.p. 30-40°C) to effect a partial separation. The more lipophilic product was columned twice more, and recrystallised from hexane to give a fluffy white solid of *methyl (1β, 2β,3α,4α,6β)-1,6-diphenylthio-3,4-isopropylidenedioxy-cyclohexane-1-carboxylate* (113)(204mg, 47%) m.p. 125.5-126.5°C (from hexane); R_F 0.67 [1.3 EtOAc - light petroleum]; ν_{max} 1725 cm^{-1} (C = 0); δ_H (400MHz) 1.37 and 1.49 (2 x 3H, 2s, CMe_2), 1.77 (2H, m,5α-,5β-H), 3.00 (1H, dd, $J_{2,3}$ = 9.5, $J_{2,1}$ = 4.5Hz, 2-H), 3.16 (1H, br d, $J_{1,2}$ = 4.5, $J_{1,6}$ = 4.0Hz, 1-H), 3.42 (1H, ddd, $J_{6,5\beta}$ = 10.5, $J_{6,5\alpha}$ = 6.5, $J_{6,1}$ = 4.0Hz, 6-H), 3.79 (3H, s, OCH_3), 4.40 (1H, dd, $J_{3,2}$ = 9.5, $J_{3,4}$ = 5.0Hz, 3-H), 4.43 (1H, m, $J_{4,5\beta}$ = 3.5Hz, 4-H), 7.26, 7.39 and 7.46 (10H, 3 x m, 2SPh); δ_C 26.14 and 28.66 (2q, $C(CH_3)_2$), 29.47 (t,5-C), 43.04 (d,1-C), 50.10(d, 6-C), 51.57(q,OCH_3), 53.44 (d,2-C), 74.36 and 75.25 (2d, 3-, 4-C), 108.56 (s, $C(CH_3)_2$), 128.04, 129.04 and 132.97 (3d, aromatic CH), 133.16 and 133.63 (2s, aromatic C-S), 171.23 (s, C=0); *m/z* 430 (E.I.) (M^+, 100%), 320 (17), 156(18), 153(8). (Found : C, 64.3, H, 6.2. $C_{23}H_{26}O_4S_2$ requires C, 64.2; H, 6.05%).

Double Resonance Data for (113)

Signal irradiated (Chemical shift, δ)	Observed Resonance					
	4-H	3-H	6-H	1-H	2-H	5α-,5β-H
Original resonance	m	dd	ddd	br d	dd	m
2-H (3.00)	m	d	ddd	dd	-	m
1-H (3.16)	m	dd	dd	-	d	m
6-H (3.43)	m	ddd	-	d	dd	simplified m

NOEDS data for (113)

Signal irradiated (Chemical shift, δ)	Observed nOe (% enhancement)
5α-H, 5β-H (2.30)	*o*- Ph(1%), 4-H(7%), 6-H(4%)
2-H (3.00)	*o*- Ph(4%), 3-H(2%), 6-H(7%), 1-H(6%) 5α-H(2%), CMe_2(5%)

The more hydrophilic fraction obtained from the partial chromatograhic separation was found to comprise a mixture of *methyl (2α, 3α, 4α)-3,4-isopropylidenedioxy-2-phenylthio-cyclohex-6-ene-1-carboxylate* (**114**) and *methyl (2β, 3α, 4α)-3,4-isopropylidenedioxy-2-phenylthio-cyclohex-6-ene-1-carboxylate* (**115**) in a 1:5 ratio (74mg, 22%). R_F 0.62 [1:2 EtOAc-light petroeum]. An analytical sample of each was obtained by repeated chromatography of the mixture, eluting with 20% ether-light

petroleum (b.p. 30-40°C). The less polar α-allylic sulphide (114) was eluted first as an oil, with ν_{max} 1710 cm^{-1} (C=0); δ_H 1.26 and 1.28 (2 x 3H, 2s, CMe_2), 2.20 (1H, ddd, J_{gem} = 13.5, $J_{5\beta,4}$ = 5.5, $J_{5\beta,6}$ = 3.5 Hz, 5β-H), 2.27 (1H, br m, [obscured by 5β-H], 5α-H), 3.77 (3H, s, OCH_3). 4.36 (1H, dd, $J_{2,3}$ = 3.5, $J_{2,5\alpha}$ = 3.0 Hz, 2-H), 4.65 (1H, dd, $J_{3,4}$ = 6.5, $J_{3,2}$ = 3.5Hz, 3-H), 4.84 (1H, br ddd, $J_{4,5\alpha}$ = 7.0, $J_{4,3}$ = 6.5, $J_{4,5\beta}$ = 5.5 Hz, 4-H), 7.01 (1H, br d, $J_{6,5\beta}$ = 3.5Hz, 6-H), 7.25-7.60 (5H, m, SPh); δ_C 24.38 and 26.31 (2q, $C(CH_3)_2$), 28.61 (t,5-C), 43.66 (d,2-C), 52.03 (q,$OCH_3)_2$), 72.41 (d,3-C), 76.81 (d, 4-C), 108.25 (s, $C(CH_3)$, 127.63, and 129.16 (2d, aromatic CH), 129.68 (s, 1-C), 132.21 (d, aromatic CH), 133.75 (s, aromatic C-S), 140.59 (d, 6-C), 165.60(s, C=0); *m/z* (E.I.) 320 (M^+, 320.1073. $C_{17}H_{20}O_4S$ requires M, 320.1080, 78%), 262(30), 153(74), 110(100). The more polar β-allylic sulphide (115) was also obtained as a colourless oil, with ν_{max} 1710cm^{-1} (C=0); δ_H 1.27 and 1.30(2 x 3H, 2s, CMe_2), 2.50 (1H, ddd, J_{gem} = 18.0, $J_{5\beta,4}$ = 4.0, $J_{5\beta,6}$ = 3.5Hz, 5β-H), 2.57 (1H, ddd, J_{gem} = 18.0, $J_{5\alpha,6}$ = 7.0, $J_{5\alpha,4}$ = 2.0Hz, 5α-H), 3.78 (3H, s, OCH_3, 4.52 (1H, d, $J_{2,3}$ = 1.5Hz, 2-H), 4.62 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 1.5Hz, 3-H), 4.65 (1H, br m, 4-H), 7.17 (1H, dd, $J_{6,5\alpha}$ = 7.0, $J_{6,5\beta}$ = 3.5, 6-H), 7.20-7.52 (5H, m, SPh); *m/z* (E.I.) 320 (M^+, 320.1084.$C_{17}H_{20}O_4S$ requires M, 320.1080,100%), 262(52), 218(40).

Methyl (1β,2β,3α,4α)-3,4-isopropylidenedioxy-2-phenylthio-cyclohex-5-ene-1-carboxylate (106) - To a solution of the diene ester (81) (210mg, 1mmol) and thiophenol (0.21cm^3, 2 mmol) in chloroform (1cm^3) was added triethylamine (10 μL). After stirring for 1h. at ambient temperature the reaction mixture was diluted with ether, washed successively with 5% aqueous NaOH, water and bring, and dried (Na_2SO_4). The solvents were removed under reduced pressure to leave a white residue which was purified by flash chromatography [eluting with 1:6 ether-light petroleum (b.p. 30-40°C)] to yield the title compound as a white solid (188mg, 59%). Recrystallisation of a sample from petroleum ether gave long, transluent crystals m.p. 100.5 - 101°C; R_F 0.34 [20% Et_2O - petroleum ether]; ν_{max} 1735cm^{-1} (C=0); δ_H 1.38 and 1.39 (2 x

3H, 2s, CMe_2), 3.72 (4H, s and m, OCH_3, 1-H), 3.81 (1H, dd, $J_{2,1}$ = 5.0, $J_{2,3}$ = 5.5Hz, 2-H), 4.62 (1H, dd, $J_{3,2}$ = 5.5, $J_{3,4}$ = 5.5Hz, 3-H), 4.76 (1H, br m, 4-H), 5.91 (1H, ddd, $J_{5,6}$ = 10.0, $J_{5,4}$ = 3.0, $J_{5,1}$ = 2.5Hz, 5-H), 6.12 (1H, dd, $J_{6,5}$ = 10.0, $J_{6,1}$ = 3.0Hz, 6-H), 6.92 and 7.41 (5H, 2m, SPh); δ_C 26.39 and 27.77 (2q, $C(CH_3)_2$), 42.34 (d,1-C), 50.21 (d,2-C), 52.08 (q, OCH_3), 71.44 and 75.23 (2d, 3-, 4-C), 109.73 (s, $C(CH_3)_2$), 125.64, 127.64, 128.07, 129.08 and 132.33 (5d, 5-, 6-, aromatic CH), 134.48 (s, aromatic C-S), 171.65 (s, C=0); *m/z* (E.I.) 320 (M^+, 100%), 245(23) (Found : C, 63.9; H, 6.35. $C_{17}H_{20}O_4S$ requires C, 63.7; H, 6.3 %)

Double Resonance Data for (106)

Signal irradiated (Chemical shift, δ)	Resonance observed				
	6-H	5-H	4-H	3-H	2-H
Original signal	dd	ddd	br m	dd	dd
6-H (6.12)		dd	resolved	dd	dd
4-H (4.76)	dd	dd		br d	dd

NOEDS data for (106)

Signal irradiated	Observed nOe (% enhancement)					
(Chemical shift, δ)	*o*-H	*m*/*p*-H	6-H	5-H	3-/4-H	2-H	1-H
aromatic *o*-H (7.40)		10			1	6	
6-H (6.11)				9			6
5-H (5.77)			20		5		
3-/4-H (4.70)	2			15		11	
2-H (4.02)	6				5		18
1-H (3.74)			13			11	

Methyl (1β,2β,3α,4α,)-3,4-isopropylidenedioxy-2-phenylthio-cyclohex-5-ene-1-carboxylate (116) - The phenylsulphide (106) (127mg, 0.40 mmol) was heated at 40°C with *m*-CPBA (0.22g, 1.29 mmol) in dichloromethane (6cm^3). T.l.c. analysis after 1h. showed that all the starting material had been converted to two spots of product R_F 0.59 and 0.48 [50% EtOAc-light petroleum]. After 3 days only the more hydrohilic product was detected. The reaction was diluted with dichoromethane, washed successively with 10% aq. sodium sulphite, saturated sodium hydrogen carbonate solution, and brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield a pale yellow oil, which solidified on standing to give the phenylsulphone (116) as a cream-coloured solid [125mg, 89%], m.p. 108-109°C (from ether-light petroleum); R_F 0.48 [50% EtOAc-light petroleum]; ν_{max} 1730 (C=0), 1655(C=C), 1310 (SO_2 asymmetric), 1150cm^{-1} (SO_2 symmetric); δ_H 1.06 and 1.31 (2 x 3H, 2s, CMe_2), 3.55 (1H, dd, $J_{2,3}$ = 9.0, $J_{2,1}$ = 4.5Hz, 2-H), 3.79 (3H, s, OCH_3), 3.87 (1H, br d, 1-H), 4.76 (1H, br dd, 4-H), 5.18 (1H, dd, $J_{3,2}$ = 8.5, $J_{3,4H}$ = 6.5Hz, 3-H), 6.01 (1H, ddd, $J_{5,6}$ = 9.5, $J_{5,4}$ = 3.5, $J_{5,1}$ = 1.5Hz, 5-H), 6.12 (1H, ddd, $J_{6,5}$ = 9.5, $J_{6,1}$ = 5.5, $J_{6,4}$ = 1.0Hz, 6-H), 7.47-7.68 and 7.95 (5H, 2m, SO_2Ph); δ_C 24.49 and 26.80 (2q, $C(CH_3)_2$),

42.32(d, 1-C), 52.26(q, OCH_3), 65.58(d, 2-C), 72.81 and 73.04 (2d,3-, 4-C), 109.07(s,$C(CH_3)_2$), 127.01, 128.14, 129.35 and 135.02 (4d, aromatic CH, 5-, 6-C), 142.99 (s, aromatic C-S), 170.08 (s, C=O); m/z 353 (MH^+, 7%), 337(4), 295(54), 157(100). (Found : C,57.4; H, 5.5. $C_{17}H_{20}O_6S$ requires C, 57.95; H, 5.7 %).

(3α,4α) 3,4-isopropylidenedioxy-cyclohexa-1,5-diene-1-carboxylic acid (117) - The diene ester (212 mg, 1.0 mmol) was stirred in acetone-water (1:9, 20cm^3) at ambient temperature, and to the cloudy white solution was added pig liver esterase (300 μL, 120U). 0.05M, pH7 phosphate buffer [$Na_2HPO_4.12H_2O$ (3.201g), KH_2PO_4 (0.484g) in 250ml of H_2O] was added periodically to maintain the reaction at pH7. After 2h. the starting material had reacted completely (t.l.c., 50% EtOAc-hexane), the solvents were evaporated and the white residue taken up in 50cm^3 of water. This was acidified to pH3 with 2M HCl and the product extracted with ethyl acetate. Reacidification of the aqueous portion and extraction was carried out twice more, and the combined extracts dried ($MgSO_4$) and evaporated to a yellow oily solid. This was taken up in chloroform and washed with water, dried ($MgSO_4$) and concentrated to a yellow oil (195mg, 99%) which solidified on standing. A sample was purified by flash chromatography eluting with 70:30:1 hexane-ethyl acetate-formic acid to furnish a white solid (m.p 92-94°C). R_F 0.31 [70:30:1, hexane-EtOAc-HCO_2H]; ν_{max} 3850-2270(COOH), 1695cm^{-1} (C=O); δ_H 1.41 and 1.43 (2 x 3H, 2s, CMe_2), 4.66 (1H, dd, $J_{4,3}$ = 9.0, $J_{4,5}$ = 4.0Hz, 4-H), 4.85 (1H, dd, $J_{3,4}$ = 9.0, $J_{3,2}$ = 3.5Hz, 3-H), 6.07 (1H, dd, $J_{5,6}$ = 10.0, $J_{5,4}$ = 4.0Hz, 5-H), 6.54 (1H, d, $J_{6,5}$ = 10.0Hz, 6-H), 6.88 (1H, br s, COOH), 7.00 (1H, dd, $J_{2,3}$ = 3.5, $J_{2,6}$ = 1.0Hz, 2-H); *m/z* 139 (MH^+, 100%), 138(32), 121(30). (Found : C, 60.9; H, 6.2. $C_{10}H_{12}O_4$ requires C, 61.2; H, 6.1 %).

1α, 2α,-epoxy- 3α,4α -isopropylidenedioxy-cyclohex-5-ene-1β-carboxylic acid (119) - Pig liver esterase (60U) was added to a stirred solution of the epoxy ester (88) (89mg, 0.39 mmol) in acetone water (1:9,5cm^3). The reaction was maintained at pH7 by the periodic addition of 0.05M phosphate buffer. After 3 days the solution was shaken with dichloromethane, and the organic extracts dried ($MgSO_4$) and evaporated to leave unreacted (88) (39mg). This exhibited optical activity, $[\alpha]_D$ + 12.8° (*c* 0.17, $CHCl_3$). The aqueous portion was acidified to pH2 with 0.1M HCl, and the product extracted with dichloromethane. The dried extracts were concentrated under reduced pressure to yield the title compound as a white solid (8mg, corrected yield 19%). m.p. 93-95.5°C; R_F 0.20 [60:40:1 light petroleum-EtOAc-HCO_2H]; ν_{max} 3600-2340 (CO_2H), 1705 cm^{-1} (C=0); δ_H 1.40 (6H, 2s, CMe_2), 3.95 (1H, d, $J_{2,3}$ = 2.0Hz, 2-H), 4.51 (1H, ddd, $J_{4,3}$ = 7.0, $J_{4,5}$ = 2.5, $J_{4,6}$ = 2.0Hz, 4-H), 4.81 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 2.0Hz, 3-H), 5.91 (1H, dd, $J_{5,6}$ = 10.0, $J_{5,4}$ = 2.5Hz, 5-H), 6.38 (1H, dd, $J_{6,5}$ = 10.0, $J_{6,4}$ = 2.0Hz, 6-H); *m/z* (-ve FAB, H_2O-glycerol) 212 (M^+,7%), 167(12), 109(13).

(3α,4α,5β,6β,)-3,4-dihydroxy-5,6-epoxy-cyclohex-1-ene-1-carboxylic acid (121) - A solution of the protected hydroxy acid (120) (18mg, 0.09 mmol) in methanol (2cm^3) was stirred with Dowex 50W-X8 (H^+) ion exchange resin at ambient temperature for 24h. The resin was filtered off and the solvent evaporated from the filtrate to furnish the title compound as a yellow oil (11mg,72%) δ_H ($CDCl_3$-D_2O) 3.76 (1H, dd, $J_{4,5}$ = 8.0, $J_{4,3}$ = 4.0Hz, 4-H), 4.00 (1H, dd, $J_{5,4}$ = 8.0, $J_{5,6}$ = 5.0Hz, 5-H), 4.34 (1H, d, $J_{6,5}$ = 5.0Hz, 6-H), 4.42 (1H, dd, $J_{3,2}$ = 4.0, $J_{3,4}$ = 4.0Hz, 3-H), 6.82 (1H, d, $J_{2,3}$ = 4.0Hz, 2-H).

Methyl (3α,4α,5β,6β,)-5,6-epoxy-3,4-isopropylidenedioxy-cyclohex-1-ene-1-carboxylate (**87**) - The diene acid (**81**) (106mg, 0.5 mmol) in chloroform ($3cm^3$) was stirred overnight with *m*-CPBA (100mg, 0.5 mmol) at ambient temperature. Removal of the white precipitate of *m*-chlorobenzoic acid by filtration, and evaporation of the filtrate under reduced pressure afforded a white gum (110mg) of *(3α,4α,5β,6β,)-5,6-epoxy-3,4-isopropylidenedioxy-cyclohex-1-ene-1-carboxylic acid* (120) R_F 0.38 [60:40:1 light petroleum-EtOAc-HCO_2H]; δ_H (200MHz) 3.70 (1H, dd, $J_{5,6}$ = 3.5, $J_{5,4}$ = 2.5 Hz, 5-H), 4.01 (1H, dd, $J_{6,5}$ = 3.5, $J_{6,2H}$ = 1.5Hz, 6-H), 4.62 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 2.5Hz, 3-H), 4.83 (1H, br dd, $J_{4,3}$ = 7.0, $J_{4,5}$ = 2.5Hz, 4-H), 6.98 (1H, dd, $J_{2,3}$ = 2.5, $J_{2,6}$ = 1.5Hz, 2-H), 8.70 (1H, br s, CO_2H). This was contaminated with *ca.* 10% *m*-chlorobenzoic acid, but was reacted without further purification. The gum (110mg) was dissolved in ether ($5cm^3$) and treated with an etherial solution of diazomethane. The ensuing reaction was monitored frequently the t.l.c., and, when complete, the solvent was allowed to evaporate. The resulting yellow oil was chromatographed with 10% EtOAc - light petroleum to yield the title compound as a colourless oil (69mg, 56% overall yield) R_F 0.28 [10% EtOAc-light petroleum]; ν_{max} $1720cm^{-1}$ (C=0); δ_H1.37 and 1.41 (2 x 3H, 2s, CMe_2), 3.67 (1H, dd, $J_{5,6}$ = 3.5, $J_{5,4}$ = 2.0Hz, 5-H), 3.83 (3H, s, OCH_3), 4.00 (1H, ddd, $J_{6,5}$ = 3.5, $J_{6,2}$ = 1.5, $J_{6,4}$ = 0.5Hz, 6-H), 4.58 (1H, dd, $J_{3,4}$ = 7.0, $J_{3,2}$ = 2.5Hz, 3-H), 4.81 (1H, m, $J_{4,3}$ = 7.0, $J_{4,5}$ = 2.0, $J_{4,2}$ = 0.5, $J_{4,6}$ = 0.5Hz, 4-H), 6.81 (1H, ddd, $J_{2,3}$ = 2.5, $J_{2,6}$ = 1.5, $J_{2,4}$ = 0.5Hz, 2-H); δ_C 25.94 and 27.79 (2q, $C(CH_3)_2$), 46.05 (d, 6-C), 49.24(d, 5-C), 52.22 (q, OCH_3), 70.81 and 71.24 (2d, 3-, 4-C), 111.00 (s, $C(CH_3)_2$), 127.47 (s, 1-C), 139.99 (d, 2-C), 165.39 (s, C=0); *m/z* 211 (M^+-15,31%), 169(100), 137(58) (Found : C, 58.4; H, 6.25. $C_{11}H_{14}O_5$ requires C, 58.4; H, 6.2%).

1-carbomethoxy-2,3-endo-epoxy-3,4-exo-isopropylidenedioxy-8,9-diazabicyclo [4,3,0] non-8-ene (122) - The epoxy acid (120)-*m*-chlorobenzoic acid mixture (279mg, *ca.* 0.6 mmol of (120) by ^{1}H n.m.r.) was dissolved in diethyl ether (5cm^3) and swirled vigorously whilst an etherial solution of diazomethane was added dropwise. Addition was continued until the yellow colour persisted indicating an excess of diazomethane. The excess reagent and ether were allowed to evaporate to leave a pale yellow oil (331mg). This was columned with 20% ethyl acetate-light petroleum, to furnish the title compound as a white solid (116mg, *ca.* 70%) m.p. 115-116°C; R_F 0.48 [50% EtOAc-light petroleum]; ν_{max} 1725cm^{-1} (C=0); δ_H 1.12 and 1.26 (2 x 3H, 2s, CMe_2), 2.66 (1H, ddd, $J_{6,7endo}$ = 11.5, $J_{6,7exo}$ = 10.0, $J_{6,5}$ = 3.0 Hz, 6-H), 2.92 (1H, ddd, $J_{3,2}$ = 3.5, $J_{3,4}$ = 1.0, $J_{3,5}$ = 1.0Hz, 3-H), 3.33 (3H, s, OCH_3), 3.60 (1H, dd, J_{gem} = 16.5, $J_{7endo,6}$ = 11.5Hz, 7endo-H), 3.66 (1H, ddd, $J_{5,4}$ = 5.5, $J_{5,6}$ = 3.0, $J_{5,3}$ = 1.0 Hz, 5-H), 3.78 (1H, br d, $J_{4,5}$ = 5.5Hz, 4-H), 3.97 (1H, br d, $J_{2,3}$ = 3.5Hz, 2-H), 4.16 (1H, dd, J_{gem} = 16.5, $J_{7exo,6}$ = 10.0Hz, 7exo-H); δ_C 26.13 and 27.81 (2q, $C(CH_3)_2$), 35.79 (d, 6-C), 52.78 (q, OCH_3), 53.20 and 54.86 (2d, 2-, 3-C), 69.19 and 71.56 (2d, 4-, 5-C), 79.37 (t, 7-C), 91.89(s, 1-C), 109.24/s, CMe_2), 169.65 (s,C=0); *m/z* 269 (MH^+,100%), 225(21), 183(43),151(85) (Found : C, 53.3; H, 6.2. N, 10.1. $C_{12}H_{16}O_5N_2$ requires C, 53.7; H, 6.0; N, 10.45%).

Double Resonance Data for (122)

Signal irradiated (Chemical shift, δ)	Resonance observed						
	7_{exo}-H	2-H	4-H	5-H	7_{endo}-H	3-H	6-H
Original signal	dd	br d	br dd	ddd	dd	br d	br m
7exo-H (4.16)	dd	br s	br dd	dd	dd	-	br m
3-H (2.92)	-	br d	ddd	ddd	d	br d	br dd

NOEDS data for (122)

Signal irradiated	Observed nOe (% Enhancement)							
(Chemical shift, δ)	7exo-H	2-H	4-H	5-H	7endo-H	3-H	6-H	CMe_2
7endo-H(3.60)	23			15	-		16	
3-H (2.92)		7.5	3			-		
6-H (2.66)	12			15		-		
CMe_2 (1.26)			4	3		3	6	-

Methyl (3α,4α,5β,6α)-6-fluoro-3,4,5-trihydroxy-cyclohex-1-ene-1-carboxylate (Methyl Fluoroshikimate) (124) - The epoxy ester (87) (38mg, 0.18 mmol) in dichloromethane ($1cm^3$) was stirred at 0°C in a polythene tube, and anhydrous hydrogen fluoride-pyridine (*ca.* 70% HF, $0.5cm^3$) added dropwise *via* a polythene pipette. After 15 minutes the reaction mixture was added dropwise to aqueous calcium acetate (0.125g in $5cm^3$), and the resulting fine white precipitate removed by filtration through a short pad of celite. The filtrate was shaken with dichloromethane (2 x $2cm^3$) and the combined extracts washed once with brine, dried (Na_2SO_4) and concentrated to a colourless oil (1.4mg, 3%). ^1H n.m.r. showed this to be a 4:1 mixture of two fluoro alcohols (125) and (126) : *methyl (3α,4α,5β,6α)-6-fluoro-5-hydroxy-3,4-isopropylidenedioxy-cyclohex-1-ene-1-carboxylate* (125), the major isomer, has δ_H 1.26 and 1.49 (2 x 3H, 2s, CMe_2), 3.85 (3H, s, OCH_3), 4.27 (1H, ddd, $J_{5,F}$ = 10.5, $J_{5,4}$ = 6.5, $J_{5,6}$ = 5.5 Hz, 5-H), 4.30 (1H, dd, [partially obscured by 5-H] $J_{3,2}$ = 3.5Hz, 3-H), 4.75 (1H, ddd, $J_{4,5}$ = 6.5, $J_{4,3}$ = 2.5, $J_{4,F}$ = 2.0 Hz, 4-H) 5.25 (1H, br ddd, $J_{6,F}$ = 46.0, $J_{6,5}$ = 5.5, $J_{6,2}$ = 1.0Hz, 6-H) 6.94 (1H, ddd, $J_{2,3}$ = 3.5, $J_{2,F}$ = 2.0, $J_{2,6}$ = 1.0 Hz, 2-H); *methyl (3α,4α,5α,6β)-5-fluoro-6-hydroxy-3,4-isopropylidenedioxy-cyclohex-1-ene-1-carboxylate* (126), the minor isomer has δ_H 1.44 (6H, s, CMe_2), 3.85 (3H, s, OCH_3), 4.41 (1H, br ddd, $J_{4,F}$ = 8.0, $J_{4,3}$ = 6.5, $J_{4,5}$ = 1.5Hz, 4-H), 4.59

(1H, br dd, $J_{6,F}$ = 6.5, $J_{6,5}$ = 2.5Hz, 6-H), 4.81 (1H, ddd, $J_{3,4}$ = 6.5, $J_{3,2}$ = 2.0, $J_{3,F}$ = 1.0 Hz, 3-H), 5.49 (1H, br dd, $J_{5,F}$ = 48.0, $J_{5,6}$ = 2.5Hz, 5-H), 6.83 (1H, ddd, $J_{2,F}$ = 2.5, $J_{2,3}$ = 2.0, $J_{2,6}$ = 1.0Hz, 2-H). The washings and aqueous phase were combined and lyophilised, and the resulting white solid flash chromatographed on silica with 10% methanol-chloroform to furnish the title compound (**124**) as a colourless oil (17mg, 49%). R_F 0.33 [10% MeOH-$CHCl_3$]; ν_{max} 3720-3060(OH), 1710 cm^{-1} (C=0); δ_H (400mHz) 2.70 (3H, br s, 3OH) 3.69 (1H, dd, $J_{4,5}$ = 9.0, $J_{4,3}$ = 4.0Hz, 4-H), 3.82 (3H, s, OCH_3), 4.23 (1H, ddd, $J_{5,F}$ = 17.0, $J_{5,4}$ = 9.0, $J_{5,6}$ = 6.0Hz, 5-H), 4.49 (1H, br dd, $J_{3,2}$ = 5.0, $J_{3,4}$ = 4.0Hz, 3-H), 5.23, (1H, br dd, $J_{6,F}$ = 48.0, $J_{6,5}$ = 6.0Hz, 6-H), 6.95 (1H, dd, $J_{2,3}$ = 5.0, $J_{2,6}$ = 1.0Hz, 2-H), δ_C (CD_3OD) 52.97 (q, OCH_3), 66.70 (dd, $J_{2,F}$ = 2.0, 3-C), 70.23 (dd, $J_{4,F}$ = 7.7Hz, 4-C), 73.41 (dd, $J_{5,F}$ = 21.2Hz, 5-C), 90.15 (dd, $J_{6,F}$ = 173.2Hz, 6-C), 130.67, (d, $J_{1,F}$ = 18.7Hz, 1-C), 142.19 (dd, $J_{2,F}$ = 5.5Hz, 2-C), 167.40 (s,C=0); *m/z* (C.I. ammonia) 224 (MNH_4^+, 224.0932.$C_8H_{15}O_5NF$ requires 224.0934, 47%), 204(14), 188(15), 80(100).

Methyl (3α, 4α, 5β, 6α,)-6-isocyano-3,4-isopropylidenedioxy-5-trimethylsilyloxy-cyclohex-1-ene-1-carboxylate (128) - A solution of epoxy ester (87) (4.2mg, 0.018 mmol), trimethylsilyl cyosanide (9.4 μL, 0.071 mmol) and dry zinc iodide (*ca.* 2mg) in dry dichloromethane (1.5cm^3) was refluxed under nitrogen for 1.5h. The reaction mixture was then allowed to cool and water(0.6cm^3) added upon which the solution turned deep red in colour. The organic portion was separated off and the aqueous phase extracted twice with dichloromethane. The combined organic portions were dried ($MgSO_4$) and the solvent removed under reduced pressure to leave a yellow oil (3.4mg 53%). This was purified by chromatography (gradient elution with light petroleum to 30%EtOAc-light petroleum) to yield the title compound as a colourless oil (0.3mg, 5%). R_F 0.58 [50% EtOAc-petroleum ether]; ν_{max} = 1720cm^{-1} (C=0); δ_H 0.18 (9H, m, $SiMe_3$), 1.38 and 1.47 (2 x 3H, 2s, CMe_2), 3.81 (3H, s, OCH_3), 4.38 (1H, br dd, $J_{4,5}$ = 11.0, $J_{4,3}$ = 1.5Hz, 4-H), 4.40, (1H, dd, $J_{6,5}$ = 6.5, $J_{6,N}$ = 2.0Hz, 6-H), 4.64 (1H, br m, $J_{5,4}$ = 11.0, $J_{5,6}$ = 6.5, $J_{5,N}$ = 2.5Hz, 5-H), 4.76 (1H, dd, $J_{3,2}$ = 2.5, $J_{3,4}$ = 1.5Hz, 3-H), 6.69 (1H, d, $J_{2,3}$ = 2.5Hz, 2-H); *m/z* 299 (MH^+-27,2%), 164(100). Insufficient sample was available for C,H and N analysis.

Methyl(5β)-5-hydroxy-cyclohexa-1,3-diene-1-carboxylate (129) - Freshly distilled 1,1,1,3,3,3-hexamethyldisilazane (2.8cm^3, 13.3 mmol) in dry THF (10cm^3) was stirred in a Nitrogen environment and cooled in a bath of ethyl acetate-liquid nitrogen. *n*-Butyl lithium (13.3 mmol) was added slowly *via* a syringe and after 30 mins. a solution of the adducts (77) (2g, 13.0 mmol) in THF (30cm^3) was added slowly from a dropping funnel. The temperature was allowed to rise slowly and the reaction quenched at -15°C with saturated aqueous ammonium choride. A white solid of lithium chloride was filtered off and the organic layer separated from the aqueous phase. The latter was extracted with chloroform (x3) and the extracts combined with the organic phase. The solvents were then removed under reduced pressure and the residue taken up in chloroform (*ca.* 50cm^3). This was washed with brine, dried ($MgSO_4$) and evaporated to a yellow oil. Purification of this oil by flash

chromatography (eluting with 1:2 EtOAc-light petroleum) afforded the hydroxy diene (129) as a colourless oil (1.78g, 89%). R_F 0.36 [1:2 EtOAc-light petroleum]; ν_{max} = 3600-3300 (OH), 1700cm^{-1} (C=0), 1620,1590cm^{-1} (C=C); δ_H (100 MHz) 2.67 (1H, dd [partly obscured by 6β-H] J_{gem} = 16.0, $J_{6\alpha,5}$ = 8.0 Hz, 6α-H), 2.76 (1H, br dd, J_{gem} = 16.0Hz, $J_{6\beta,5}$ = 2.0Hz, 6β-H), 3.25 (1H, br s, OH), 3.73 (3H, s, OCH_3), 4.35 (1H, br m, 5-H), 6.16 (2H, m, 3-, 4-H), 7.04 (1H, br m, 2-H); *m/z* (E.I.) 154(M^+, 1%), 139(34), 122(24), 95(100).

Methyl (5β)-5-(t-butyldimethylsilyloxy)-cyclohexa-1,3-diene-1-carboxylate (130) - To 2,6-lutidine (3cm^3, 25.7 mmol) under nitrogen at 0°C was added *t*-butyldimethylsilyl trifluoromethanesulphonate (2.5cm^3, 10 mmol). After 20 mins. a solution of the hydroxy diene (129) (1.54g, 10 mmol) in dry CH_2Cl_2(10cm^3) was added slowly. The solution turned orange upon addition, and stirring was continued for a further 30 mins. before the reaction was poured into iced saturated sodium hydrogen carbonate solution. The organic phase was separated and washed with 2M HCl, saturated sodium hydrogen carbonate solution and brine, dried ($MgSO_4$) and concentrated to an amber-coloured oil (2.18g,81%). R_F 0.56 [10% EtOAc-light petroleum]. A sample was purified using a chromatatron (2mm SiO_2 plate, eluting with 3% EtOAc-light petroleum) to obtain a colourless oil. ν_{max} = 1705 (C=0), 1635, 1575cm^{-1} (C=C); δ_H 0.20 (6H, s, $SiMe_2$), 1.00 (9H, s, *t*-Bu), 2.63 (2H, m, 6α-H and 6β-H), 3.77 (3H, s, OCH_3), 4.50 (1H, ddd, $J_{5,6\beta}$ = 10.0, $J_{5,6\alpha}$ = 7.5Hz, $J_{5,4}$ = 2.0Hz, 5-H), 6.07 (2H, m, 3-, 4-H). 7.00 (1H, m, 2-H); δ_C 1.63 (q, $Si(CH_3)_2$), 22.75 (s, $SiC(CH_3)_3$), 30.39 (q, $SiC(CH_3)_3$), 36.14 (d, 6-C), 58.18 (q, OCH_3), 70.05 (d, 5-C), 128.12 (d, 4-C), 131.86 (s, 1-C), 136.36 (d, 3-C), 140.37 (d, 2-C), 172.06 (s, C=0); *m/z* 267 (M^+-1, 100%), 209(38), 135(46), 75(100) (Found : C, 62.7; H, 9.1. Calc. for $C_{14}H_{24}O_3Si$: C, 62.7; H, 9.0%).

Epoxidation of the Hydroxy Diene (129) - To a solution of the hydroxy diene (129) (1.92g, 12.47 mmol) in dichloromethane (30cm^3) at ambient temperature was added

m-CPBA (2.37g, 13.73 mmol), and the reaction stirred for 12h. A white precipitate of *m*-chlorobenzoic acid was removed by filtration and the filtrate washed with 10% aqueous sodium sulphite, saturated sodium hydrogen carbonate solution and brine, dried ($MgSO_4$) and the solvent evaporated to leave a yellow oil (1.33g, 63%). A sample was distilled (b.p. 174°C, 0.3mm, lit.,[118], 150°C, 0.2mm) but extensive resinification of the residue occured. R_F 0.34 [50% EtOAc-light petroleum]; ν_{max} = 3600-3180 (OH), 1690cm^{-1} (C=0); *m/z* 171(MH^+, 5%), 153(3), 89(100) (Found : C, 56.6; H, 5.6. calc. for $C_8H_{10}O_4$: C, 56.5; H, 5.9%). This was shown by ^1H n.m.r. to be a 15:4 mixture of *cis*- and *trans*- epoxides : *methyl (3β,4β,5β,)-3,4-epoxy-5-hydroxy-cyclohex-1-ene-1-carboxylate* (131) has δ_H 2.14 (1H, ddd, J_{gem} = 16.5, $J_{6\beta,5}$ = 10.0, $J_{6\beta,2}$ = 3.5Hz, 6β-H), 2.90 (1H, ddd, J_{gem} = 16.5 $J_{6\alpha,5}$ = 6.5, $J_{6\alpha,4}$ = 2.0Hz, 6α-H). 3.53 (1H, dd, $J_{3,4}$ = 4.5, $J_{3,2}$ = 4.0Hz, 3-H), 3.68 (1H, br m, 4-H), 3.77 (4H, br s, OCH_3 and OH), 4.15 (1H, ddd, $J_{5,6\beta}$ = 10.0, $J_{5,6\alpha}$ = 6.5, $J_{5,4}$ = 1.0Hz, 5-H), 7.02 (1H, dd, $J_{2,3}$ = 4.0, $J_{2,6\beta}$ = 3.5Hz, 2-H)

Double Resonance Data for (131)

Signal irradiated	Observed resonance				
(Chemical shift, δ)	5-H	4-H	3-H	6α-H	6β-H
Original resonance	ddd	br m	dd	ddd	ddd
5-H (4.15)	-	br d	dd	dd	dd
6α-H (2.90)	br d	br d	dd	-	dd
6β-H (2.14)	br d	br m	dd	br dd	-

Methyl (3α, 4α, 5β)-3,4-epoxy-5-hydroxy-cyclohex-1-ene-1-carboxylate (132) - has δ_H 2.30 (1H, ddd, J_{gem} = 17.5, $J_{6\alpha,5}$ = 5.0, $J_{6\alpha,2}$ = 3.5Hz, 6α-H), 2.80 (1H, ddd, J_{gem} = 17.5, $J_{6\beta,5}$ = 2.5, $J_{6\beta,4}$ = 2.0Hz, 6β-H), 3.50 (1H, dd, $J_{3,2}$ = 4.0, $J_{3,4}$ =

3.5Hz, 3-H), 3.61 (1H, br m, 4-H), 3.77 (4H, br s, OCH_3 and OH), 4.58 (1H, ddd, $J_{5,6\alpha}$ = 5.0, $J_{5,6\beta}$ = 2.5, $J_{5,4}$ = 2.0Hz, 5-H), 7.14 (1H, dd, $J_{2,3}$ = 4.0, $J_{2,6\alpha}$ = 3.5Hz, 2-H).

Epoxidation of the Silyloxy Diene (**130**) - To a solution of silyloxy diene (**130**) (1.10g, 4.10 mmol) in dichloromethane (15cm^3) was added *m*-CPBA (0.78g, 4.52 mmol), and the solution stirred at 40°C for 2h. Dichloromethane (20cm^3) was added and the diluted reaction mixture washed with 10% aqueous sodium sulphite, saturated sodium hydrogen carbonate solution and brine, dried (Na_2SO_4) and concentrated to a yellow oil (1.08g,93%) ^{1}H n.m.r. analysis of the crude product showed it to be a 23:4 mixture of *trans*- and *cis*- epoxy silyl ethers*. A sample was chromatographed with 3% EtOAc-light petroleum to give *methyl (3α,4α,5β,)-5-(t-butyldimethylsilyloxy)-3,4-epoxy-cyclohex-1-ene-1-carboxylate* (**134**) as a colourless oil. R_F 0.45 [10% EtOAc- light petroleum]; ν_{max} (liquid film) 1720cm^{-1} (C=0); δ_H (400MHz) 0.07 and 0.09 (2 x 3H, 2s, $SiMe_2$), 0.85 (9H, s, *t*-Bu), 2.21 (1H, ddd, J_{gem} = 17.0, $J_{6\alpha,5}$ = 5.0, $J_{6\alpha,2}$ = 3.0 Hz, 6α-H), 2.66 (1H, ddd, J_{gem} = 17.0, $J_{6\beta,5}$ = 2.0, $J_{6\beta,4}$ = 2.0 Hz, 6β-H), 3.41 (1H, ddd, $J_{4,3}$ = 4.0, $J_{4,5}$ = 2.5, $J_{4,6\beta}$ = 2.0 Hz, 4-H), 3.45 (1H, dd, $J_{3,2}$ = 4.0, $J_{3,4}$ = 4.0Hz, 3-H), 3.74 (3H, s, OCH_3), 4.51 (1H, ddd, $J_{5,6\alpha}$ = 5.0, $J_{5,4}$ = 2.5, $J_{5,6\beta}$ = 2.0Hz, 5-H), 7.08 (1H, dd, $J_{2,3}$ = 4.0, $J_{2,6\alpha}$ = 3.0Hz, 2-H); δ_C 1.74 (q, $Si(CH_3)_2$), 18.26 (s, $SiC(CH_3)_3$), 25.84 (q, $SiC(CH_3)_3$), 29.60 (t, 6-C), 46.80 (d, 4-C) 51.91 (q, OMe) 56.47 (d, 3-C), 64.29 (d, 5-C), 131.16 (s, 1-C), 133.15 (d, 2-C), 166.64 (s, C=0), *m/z* 285 (MH^+, 100%), 253(67), 227(28), 153(57) (Found : C, 59.0; H, 8.4. $C_{14}H_{24}O_4Si$ requires : C, 59.2; H, 8.45%). Continued elution gave *methyl (3β,4β,5β,)-5-(t-butyldimethylsilyloxy)-3,4-epoxy-cyclohex-1-ene-1-carboxylate (133) also as a colourless oil. R_F 0.36 [10% EtOAc- light petroleum];* ν_{max} (liquid film) 1715cm^{-1} (C=0); δ_H 0.05 and 0.08 (2 x 3H, 2s, $SiMe_2$), 0.93 (9H, s, *t*- Bu),

* These compounds have been reported[20] but without accompanying experimental or spectral data.

2.22 (1H, ddd, J_{gem} = 16.5, $J_{6\beta,5}$ = 10.0, $J_{6\beta,2}$ = 3.5 Hz, 6β-H), 2.72 (1H, ddd, J_{gem} = 16.5, $J_{6\alpha,5}$ = 6.5, $J_{6\alpha,4}$ = 2.0 Hz, 6α-H), 3.46 (1H, dd, $J_{3,4}$ = 4.0, $J_{3,2}$ = 3.5 Hz, 3-H), 3.51 (1H, ddd, $J_{4,3}$ = 4.0, $J_{4,6\alpha}$ = 2.0, $J_{4,5}$ = 1.0Hz, 4-H), 3.75 (3H,s,OCH_3), 4.15 (1H, ddd, $J_{5,6\beta}$ = 10.0, $J_{5,6\alpha}$ = 6.5, $J_{5,4}$ = 1.0Hz, 5-H), 7.00 (1H, dd, $J_{2,3}$ = 3.5, $J_{2,6\beta}$ = 3.5Hz, 2-H); *m/z* 285 (MH^+, 92%), 227(100), 153(100) (Found : C, 58.7; H, 8.6. $C_{14}H_{24}O_4Si$ requires : C, 59.2; H, 8.45%).

Reaction of cis- and trans- Epoxy Alcohols (131) and (132) with Azidotrimethylsilane - A mixture of *cis-* and *trans*-epoxy alcohols (131) and (132) (200mg,1.18mmol) in dichloromethane (1cm^3) was stirred at ambient temperature with azidotrimethylsilane (16 μl, 1.21mmol). After 2 days 2M HCl (1cm^3) was added and stirring continued for 10mins. The reaction mixture was diluted with dichloromethane (3cm^3) and washed with saturated sodium hydrogen carbonate solution and brine, dried (Na_2SO_4) and the solvent removed under reduced pressure. The resulting oil (147mg, 52%) was chromatographed (gradient elution with light petroleum; 5% EtOAc-light petroleum) to give *methyl (3α,4α,5β,)-3,4-epoxy-5-trimethylsilyloxy-cyclohex-1-ene-1-carboxylate* (139) (24mg,9%) as a colourless oil. R_F 0.35 [10% EtOAc- light petroleum]; ν_{max} 1710cm^{-1} (C=0); δ_H (9H, s, $SiMe_2$), 2.22 (1H, ddd, J_{gem} = 17.5, $J_{6\alpha,5}$ = 5.0, $J_{6\alpha,2}$ = 3.0 Hz, 6α-H), 2.65 (1H, ddd, J_{gem} = 17.5, $J_{6\beta,5}$ = 2.0, $J_{6\beta,4}$ = 2.0 Hz, 6β-H), 3.40 (1H, ddd, $J_{4,3}$ = 4.5, $J_{4,5}$ = 2.5, $J_{4,6\beta}$ = 2.0 Hz, 4-H), 3.44 (1H, dd, $J_{3,4}$ = 4.5, $J_{3,2}$ = 4.0Hz, 3-H), 3.73 (3H, s, OCH_3), 4.49 (1H, ddd, $J_{5,6\alpha}$ = 5.0, $J_{5,4}$ = 2.5, $J_{5,6\beta}$ = 2.0Hz, 5-H), 7.09 (1H, dd, $J_{2,3}$ = 4.0, $J_{2,6\alpha}$ = 3.0Hz, 2-H); *m/z* 243 (MH^+, 26%), 227(23), 211(44), 153(100). Further elution with 5% EtOAc-light petroleum gave *methyl (3β,4β,5β,)-3,4-epoxy-5-trimethylsilyloxy-cyclohex-1-ene-1-carboxylate* (138) also as a colourless oil (13mg,5%). R_F 0.29 [10% EtOAc- light petroleum]; ν_{max} 1710cm^{-1} (C=0); δ_H 0.19 (9H,s,$SiMe_3$), 2.21 (1H, ddd, J_{gem} = 16.5, $J_{6\beta,5}$ = 10.0, $J_{6\beta,2}$ = 3.5 Hz, 6β-H), 2.73 (1H, ddd, J_{gem} = 16.5, $J_{6\alpha,5}$ = 6.5,

$J_{6\alpha,4}$ = 2.0Hz, 6α-H), 3.46 (1H, dd, $J_{3,2}$ = 4.0, $J_{3,4}$ = 4.0 Hz, 3-H), 3.51 (1H, br m,4-H), 3.75 (3H,s,OCH_3), 4.14 (1H, ddd, $J_{5,6\beta}$ = 10.0, $J_{5,6\alpha}$ = 6.5, $J_{5,4}$ = 1.0Hz, 5-H), 7.00 (1H, dd, $J_{2,3}$ = 4.0, $J_{2,6}$ = 4.0Hz, 2-H)

Methyl (3α,4β,5β)-3-azido-4,5-di-(trimethylsilyloxy)cyclohex-1-ene-1-carboxylate (140) - A solution of the *cis*-epoxy alcohol (131) (contaminated with 10% of (132)) (168mg, 0.99mmol) in dichloromethane ($2cm^3$) was stirred at 40°C with azidotrimethylsilane (260 μL, 1.98 mmol) and zinc iodide (630mg, 1.98 mmol). The starting material was consumed after 30 mins, and after 2h. the reaction was allowed to cool and was then diluted with dichloromethane. It was then washed with 0.1M sodium thiosulphate and brine, dried (Na_2SO_4) and concentrated to a yellow oil. Flash chromatography of this (eluting with light petroleum; 6% EtOAc-light petroleum) afforded the title compound as a colourless oil (209 mg, 66% corrected yield), which rapidly turned pink on standing. R_F* 0.71 [10% EtOAc-light petroleum]; ν_{max} 2090 (N_3), 1700cm^{-1} (C=0); δ_H 0.13 (18H, m, $2SiMe_3$), 2.64 (1H, dd, J_{gem} = 18.0, $J_{6\alpha,5}$ = 6.0Hz, 6α-H), 2.75 (1H, ddd, J_{gem} = 18.0, $J_{6\beta,5}$ = 9.0, $J_{6\beta,2}$ = 2.0 Hz, 6β-H), 3.75 (3H, s, OCH_3), 4.10 (1H, dd, $J_{4,3}$ = 2.0, $J_{4,5}$ = 2.0Hz, 4-H), 4.43 (1H, ddd, $J_{5,6\beta}$ = 9.0, $J_{5,6\alpha}$ = 6.0, $J_{5,4}$ = 2.0Hz, 5-H), 4.72 (1H, br dd, $J_{3,2}$ = 2.5, $J_{3,4}$ = 2.0Hz, 3-H), 6.92 (1H, m, 2-H); *m/z* 330 (MH^+-28,25%), 315(100), 241(29), 204(59). Accurate C,H and N analysis was not possible from this compound due to its rapid decomposition.

* When the t.l.c. plates were exposed to short wave u.v. light the spot corresponding to azide (131) turned yellow. This colour faded after approximately 10 mins. but reappeared on further exposure to u.v. light, only to fade again with time.

Double Resonance Data for (140)

Signal Irradiated	Observed Resonance					
(Chemical shift, δ)	2-H	3-H	5-H	4-H	6β-H	6α-H
Original Resonance	m	br dd	ddd	dd	ddd	dd
2-H (6.92)	-	br d	ddd	dd	dd	dd
3-H (4.72)	br dd	-	ddd	s	ddd	dd

Further elution with 10% EtOAc-light petroleum gave a second oil (19mg,5%); ν_{max} 2090 (N_3), 1700cm^{-1} (C=0). ^{1}H n.m.r. analysis showed this to be a complex mixture, but signals at 6.61p.p.m. and 6.79 p.p.m., corresponding to 2-H resonances, prove the existance of at least two other isomers.

Zinc Iodide Catalysed Reaction of the Epoxides (133) and (134) with Azidotrimethylsilane - A mixture of the epoxy silyl ethers (133) and (134) (43mg, 0.15 mmol), and azidotrimethylsilane (50μL, 0.36 mmol) in dichloromethane (3cm^3), was stirred at 40°C. Zinc iodide (*ca.* 2mg) was added, upon which a deep red colour was obtained. After 1h. the reaction mixture was allowed to cool and diluted with dichloromethane. This was then washed with 0.1M sodium thiosulphate and brine, and dried (Na_2SO_4). Evaporation of the solvent left a yellow oil which was chromatographed with 5% ethyl acetate-light petroleum to give *methyl (3α,4β,5β)-4-azido-5-(t-butyldimethylsilyloxy)-3-hydroxy-cyclohex-1-ene-1-carboxylate* (142) as a colourless oil (6mg,13%), which gradually turned pink on standing. R_F 0.35 [10% ethyl acetate-light petroleum]; ν_{max} 3570 (OH), 2100 (N_3), 1710cm^{-1} (C=0); δ_H 0.13 and 0.14 (2 x 3H, 2s, $SiMe_2$), 0.91 (9H, s, *t*-Bu), 2.32(1H, m, J_{gem} = 17.5, $J_{6\beta,5}$ = 9.5, $J_{6\beta,3}$ = 4.0, $J_{6\beta,2}$ = 3.0Hz, 6β-H), 2.73 (1H, br s, OH), 2.78 (1H, br ddd, J_{gem} = 17.5, $J_{6\alpha,5}$ = 5.0, $J_{6\alpha,4}$ = 1.5 Hz, 6α-H), 3.58 (1H, ddd, $J_{5,6\beta}$ =

9.5,$J_{5,4}$ = 9.5, $J_{5,6\alpha}$ = 5.0Hz, 5-H), 3.77 (3H, s, OCH_3), 3.94 (1H, ddd, $J_{4,5}$ = 9.5, $J_{4,3}$ = 7.5, $J_{4,6\alpha}$ = 1.5Hz, 4-H), 4.78 (1H, br m, 3-H), 7.02 (1H, dd, $J_{2,3}$ = 3.5, $J_{2,1\beta}$ = 2.5Hz, 2-H). Continued elution afforded *methyl (3β,4α,5β)-3-azido-5-(t-butyldimethylsilyloxy)-4-hydroxy-cyclohex-1-ene-1-carboxylate* (**141**) as a colourless oil (28mg,56%), which also turned pink on standing. R_F 0.24 [10% ethyl acetate - light petroleum]; ν_{max} 3060 (OH), 2090 (N_3), 1700cm^{-1} (C=0); δ_H 0.15 and 0.17 (2 x 3H, 2s, $SiMe_2$), 0.93 (9H, s, *t*-Bu), 2.55 (1H, m, J_{gem} = 17.5, $J_{6\beta,5}$ = 9.0, $J_{6\beta,2}$ = 2.5, $J_{6\alpha,4}$ = 1.5Hz, 6β-H), 2.71 (1H, ddd, $J_{4,5}$ = 9.0, $J_{4,5}$ = 9.0, $J_{4,3}$ = 4.0, $J_{4,6\beta}$ = 1.5Hz, 4-H), 2.82 (1H, br s, OH), 2.98 (1H, br dd, J_{gem} = 17.5, $J_{6\alpha,5}$ = 6.0Hz, 6α-H), 3.76 (3H, s, OCH_3), 4.09 (1H, ddd, $J_{5,6\beta}$ = 9.0, $J_{5,4}$ = 9.0, $J_{5,6\alpha}$ = 6.0Hz, 5-H) 5.13 (1H, br dd, $J_{3,2}$ = 5.0, $J_{3,4}$ = 4.0Hz, 3-H), 7.05 (1H, dd, $J_{2,3}$ = 5.0, $J_{2,6\beta}$ = 2.5Hz, 2-H).

Methyl (3α,4α,5α,)-3,4,5-trihydroxy-cyclohex-1-ene-1-carboxylate (Methyl 5-epi-shikimate) (**147**) - The hydroxy ester (**80**) (39mg, 0.17 mmol) in methanol (1cm^3) was allowed to stand for three days with Dowex 50W-X8(H^+) ion exchange resin. The resin was removed by filtration, and the filtrate evaporated under reduced pressure to furnish the title compound as a white solid, (32mg, quantitative) m.p. 113-114°C (from EtOAc) (Lit.,[122] 114°C); R_F 0.43 [ethyl formate]; ν_{max} 3600-3080 (OH), 1705cm^{-1} (C=0); δ_H ($(CO_3)_2CO$), 2.37 (1H, m, J_{gem} = 17.0, $J_{6\alpha,5}$ = 9.0, $J_{6\alpha,3}$ = 3.0, $J_{6\alpha,2}$ = 2.5Hz, 6α-H), 2.60 (1H, br ddd, J_{gem} = 17.0, $J_{6\beta,5}$ = 5.5, $J_{6\beta,3}$ = 1.0 Hz, 6β-H), 2.89 (2H, br s, 2OH), 3.71 (3H, s, OCH_3), 3.84 (1H, ddd, $J_{5,6\alpha}$ = 9.0, $J_{5,6\beta}$ = 5.5, $J_{5,4}$ = 1.0Hz, 5-H), 3.95 (1H, br s, OH), 4.01 (1H, br dd, $J_{4,3}$ = 2.5, $J_{4,5}$ = 1.0Hz, 4-H), 4.29 (1H, br m, 3-H), 6.65 (1H, dd, $J_{2,6\alpha}$ = 2.5, $J_{2,3}$ = 1.5Hz, 2-H); *m/z* 189 (MH^+, 4%), 171(100), 153(25), 139(87). (Found : C, 51.4; H, 6.5. Calc. for $C_8H_{12}O_5$: C, 51.1; H, 6.4%).

(3α,4α,5α,)-5-hydroxy-3,4-isopropylidenedioxy-cyclohex-1-ene-1-carboxylate (**148**) - To the hydroxy ester (**80**) (250mg, 1.1 mmol) in acetone-water (1:9,10cm^3) at ambient

temperature was added pig liver esterase (100μL, 186U). The reaction was maintained at pH7 with 0.1M phosphate buffer. After 5 days a further aliquot of enzyme (300 μL, 868U), was added. The reaction was left for a further 2 days, then the aqueous solution was washed once with ether, acidified to pH2 with 0.1M HCl, and the product extracted with ethyl acetate (x4). The combined extracts were dried (Na_2SO_4) and the solvent evaporated to yield the title compound (153mg, 65%) m.p. 205-206°C (from EtOAc); R_F 0.74 [ethyl formate]; ν_{max} 3510 (CO_2H, OH), 1720cm^{-1} (C=0); δ_H (CD_3OD-D_2O) 1.32 and 1.37 (2x3H, 2s, CMe_2), 2.34 (1H, m, J_{gem} = 16.5, $J_{6\alpha,5}$ = 10.0, $J_{6\alpha,2}$ = 3.0, $J_{6\alpha,3}$ = 3.0Hz, 6α-H), 2.60 (1H, br ddd, J_{gem} = 16.5, $J_{6\beta,5}$ = 5.5, $J_{6\beta,3}$ = 1.0 Hz, 6β-H), 3.90 (1H, ddd, $J_{4,3}$ = 5.0, $J_{4,5}$ = 2.5, $J_{4,2}$ = 1.0Hz, 4-H), 4.75 (1H, m, $J_{3,4}$ = 5.0, $J_{3,6\alpha}$ = 3.0, $J_{3,6\beta}$ = 1.0Hz, 3-H), 6.65 (1H, ddd, $J_{2,3}$ = 3.0, $J_{2,6\alpha}$ = 3.0, $J_{2,4}$ = 1.0Hz, 2-H); *m/z* 215 (MH^+, 9%), 211(2), 199(5), 157(100). (Found : C, 56.0; H, 6.5. $C_{10}H_{14}O_5$: C, 56.1; H, 6.5%).

(3α,4α,5α,)-3,4,5-trihydroxy-cyclohex-1-ene-1-carboxylate (5-epi-shikimic acid) (149) - (*a*) The trihydroxy ester (147) (40mg, 0.21 mmol) was partially dissolved in dioxan (1cm^3) and stirred vigorously at 0°C. 1M KOH (1cm^3) was added and the solution immediately turned yellow. Stirring was continued for 45 mins. before the solution was acidified with Dowex 50W X-8 (H^+) ion exchange resin. The dioxan was removed under reduced pressure and the resulting aqueous solution lyophilised to give a white foam. Trituration of this with acetonitrite gave the title compound as a white solid (32mg, 86%).

(*b*) The hydroxy acid (148) (30mg, 0.14 mmol) was dissolved in methanol (1cm^3) and allowed to stand overnight with Dowex 50W-X8(H^+) ion exchange resin (pre washed with methanol). The resin was filtered off and the filtrate concentrated to a colourless oil. Trituration with acetonitrile gave *5-epi*-shikimic acid as a white solid (19mg,80%) m.p. 151-152°C; R_F 0.49 [4:1:1 EtOAc-AcOH-H_2O]; ν_{max} (MeCN) 3700-3100 (OH, CO_2H), 1715cm^{-1} (C=0); δ_H ($(CD_3)_2CO$), 2.38 (1H, m, J_{gem} = 17.0, $J_{6\alpha,5}$ = 9.0, $J_{6\alpha,3}$ = 3.0, $J_{6\alpha,2}$ = 2.5Hz, 6α-H), 2.50 (1H, br ddd, J_{gem} = 17.0, $J_{6\beta,5}$ = 6.0,

$J_{6\beta,3}$ = 2.0 Hz, 6β-H), 3.20 (3H, br s, 3OH), 3.85 (1H, m, $J_{5,6\alpha}$ = 9.0, $J_{5,6\beta}$ = 6.0, $J_{5,3}$ = 2.0, $J_{5,4}$ = 2.0Hz, 5-H), 3.94 (1H, br dd, $J_{4,3}$ = 3.5, $J_{4,5}$ = 2.0Hz, 4-H), 4.29 (1H, br m, 3-H), 6.65 and 6.69 (1H, 2m, 2-H); *m/z* 171 (60%), 157(57), 139(100). (Found : C, 48.3; H, 5.7. $C_7H_{10}O_5$ requires C, 48.3; H, 5.75%).

REFERENCES

REFERENCES

1. E. Haslam, "The Shikimate Pathway," Butterworths, London, 1974.
2. "Recent Advances in Phytochemistry : The Shikimic Acid Pathway," ed. E. E. Conn, Plenum Press, New York, 1986, vol. 20.
3. J. M. Tedder, A. Nechvatal, A. W. Murray and J. Carnduff, "Basic Organic Chemistry Part 4 : Natural Products," Wiley, Chichester, 1972, pp 103-147.
4. R. Herbert, "The Biosynthesis of Secondary Metabolites," Chapman & Hall, London, 1981.
5. B. D. Davis, *J. Biol. Chem.*, 1951,191, 315.
6. (a)B. Ganem, *Tetrahedron*, 1978, 34, 3353; (b) B. A. Bohm, *Chem. Rev.*, 1965, 65, 435.
7. R. McCrindle, K. H. Overton and R. A. Raphael, *J. Chem. Soc.*, 1960, 1560.
8. E.E. Smissman, J. T. Suh, M. Oxman and R. Daniels, *J. Am. Chem. Soc.*, 1959, 81, 2909.
9. E. E. Smissman, J. T. Suh, M. Oxman and R. Daniels, *J. Am. Chem. Soc.*, 1962, 84, 1040.
10. R. McCrindle, K. H. Overton and R. H. Raphael, *Tetrahedron Lett.*, 1968, 1847.
11. R. K. Hill and G. R. Newkome, *Tetrahedron Lett.*, 1968, 1851.
12. E.E. Smissman and J. Pengman, *Tetrahedron Lett.*, 1968, 4601.
13. R. Grewe and I. Hinrichs, *Chem Ber.*, 1964, 97, 443.
14. M. M. Doshi, *Dissertation Abstr.*, 1964, 24, 3998.
15. M. Koreeda and M. A. Ciufolini, *J. Am. Chem. Soc.*, 1982, 104, 2308.
16. K. E. Coblens, V. B. Muralidharan and B. Ganem, *J. Org. Chem.*, 1982, 47, 5042.
17. M. M. Campbell, A. D. Kaye, M. Sainsbury and R. Yavarzadeh, *Tetrahedron Lett.*, 1984, 1629.
18. M. M. Campbell, M. Sainsbury and R. Yavarzadeh, *Tetrahedron*, 1984, 40, 5063.
19. M. M. Campbell, A. D. Kaye, M. Sainsbury and R. Yavarzadeh, *Tetrahedron*, 1984, 40, 2461.

20. D. Rajapaska, B. A. Keay and R. Rodrigo, *J. Can. Chem.*, 1984, **62**, 826.

21. P. A. Bartlett and L. A. McQuaid, *J. Am. Chem. Soc.*, 1984, **106**, 7854.

22. H. J. Bestmann and H. A. Heid, *Angew. Chem., Int. Ed. Engl.*, 1971, 10, 336.

23. R. Grewe and E. Vangermain, *Chem. Ber.*, 1965, **98**, 104.

24. M. Yoshikawa, Y. Ikeda, H. Kayakiri and I. Kitagawa, *Heterocycles*, 1982, 17, 209.

25. (a) G. W. J. Fleet and T. K. M. Shing, *J. Chem. Soc., Chem. Commun.*, 1983,849; (b) G. W. J. Fleet, T. K. M. Shing and S. M. Warr, *J. Chem. Soc., Perkin Trans I*, 1984, 905.

26. A. S. Mirza and A. Vasella, *Helv. Chim. Acta.*, 1984, **67**, 1562.

27. T. Suami, K. Tadano, Y. Ueno and Y. Iimura, *Chem. Lett.*, 1985, 37.

28. S. Masamune, W. Choy, J. S. Petersen and L. R. Sita, *Angew Chem., Int. Ed. Engl.*, 1985, 24, 1.

29. S. Masamune, L. A. Reed III, J. T. Davis and W. Choy, *J. Org. Chem.*, 1983, 48, 4441.

30. J. L. Pawlak and G. A. Berchtold, *J. Org. Chem.*, 1987, **52**, 1765.

31. J. H. Hoare, P. P. Policastro and G. A. Berchtold, *J. Am. Chem. Soc.*, 1983, **105**, 6264.

32. D. A. McGowan and G. A. Berchtold, *J. Org. Chem.*, 1981, **46**, 2381.

33. A. J. Birch, L. F. Kelley and D. V. Weerasuria, *J. Org. Chem.*, 1988, **53**, 278

34. B. M. R. Bandara, A. J. Birch and L. F. Kelley, *J. Org. Chem.*, 1984, **49**, 2496.

35. A. J. Birch and D. H. Williamson, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1892.

36. G. Dangschat and H.O.L. Fischer, *Biochim. Biophys. Acta.*, 1950, **4**, 199.

37. R. Grewe and W. Lorenzen, *Ber.*, 1953, **86**, 928.

38. R. Grewe, H. Büttner and G. Burmeister, *Angew Chem.*, 1957, **69**, 61.

39. J. Corse and R. E. Lundin, *J. Org. Chem.*, 1970, **35**, 1904.

40. C. D. Snyder and H. Rapoport, *J. Am. Chem. Soc.*, 1973, **95**, 7821.

41. J. Cleophax, D. Mercier and S. D. Gero, *Angew. Chem, Int. Ed. Engl.*, 1971, **10**, 652; J. Cleophax, J. Leboul, D. Mercier A. Gaudemer and S. D. Gero, *Bull. Soc.*

Chim. Fr., 1973, 2992.

42. D. E. Metzlar, "Biochemistry. The Chemical Reactions of Living Cells," Academic Press, London, 1977, International edn., pp. 315-324.
43. (a) A. D. Kaye, Ph.D. Thesis, University of Bath, 1984. (b) R. Yavarzadeh, Ph.D.Thesis, University of Bath, 1985.
44. W. G. Dauben and H. O. Krabbenhoft, *J. Am Chem. Soc.*, 1976, **98**, 1992.
45. F. Brion, *Tetrahedron Lett.*, 1982, 5299.
46. W. L. Nelson and D. R. Allen, *J. Heterocycl. Chem.*, 1972, **9**, 561.
47. J. A. Moore and E. M. Partain III, *J. Org. Chem.*, 1983, **48**, 1105.
48. M. P. Kunstmann, D. S. Tarbell and R. L. Autrey, *J. Am Chem. Soc.*, 1962, **84**, 4115.
49. I. Fleming, "Frontier Orbitals and Organic Chemical Reactions," Wiley, Chichester, 1976, p. 161.
50. For other examples of furan adducts where the *exo*-isomer is the thermodynamic product see : W. Carruthers, "Some Modern Methods of Organic Synthesis," Cambridge University Press, Cambridge, 1978, second edn., p. 190.
51. M. Schröder, *Chem. Rev.*, 1980, 80, 187.
52. (a) B. A. Keay and R. Rodrigo, *Can. J. Chem.*, 1983, **61**, 637; (b) B. A. Keay, D. Rajapaksa and R. Rodrigo, *Can. J. Chem.*, 1984, **62**, 1093.
53. J. E. Baldwin, *J. Chem. Soc., Chem. Commun.*, 1976, 734; J. E. Baldwin, J. Cutting, W. Du Pont, L. Kruse, L. Silberman and R. C. Thomas, *J. Chem. Soc., Chem. Commun.*, 1976, 736.
54. O. Mitsunobu, *Synthesis*, 1981, 1.
55. D. Y. Curtin, *Record Chem. Prog.*, 1954, **15**, 111.
56. R. J. Arhart and J. C. Martin, *J. Am Chem. Soc.*, 1972, 94, 5003.
57. D. A. Johnson in "Sulfur in Organic and Inorganic Chemistry", ed. A. Senning, Marcel Dekker, New York, 1972, vol. 2, p.61.
58. H. Goldwhite, "Introduction to Phosphorus Chemistry," Cambridge University Press, Cambridge, 1981, p. 30.

59. P. J. Stang, M. Hanack and L. R. Subramanian, *Synthesis*, 1982, 85.

60. R. L. Hansen, *J. Org. Chem.*, 1965, 29, 4322; A. Streitwieser Jr, C. L. Wilkins and E. Kiehlmann, *J. Am. Chem. Soc.*, 1968, 90, 1598; T. M. Su, W. F. Sliwinski and P. V. R. Schleyer, *J. Am. Chem. Soc.*, 1969, 91, 5386.

61. M. Karplus, *J. Chem. Phys.*, 1959, 30, 11.

62. K. Tori, T. Komeno and T. Nakagawa, *J. Org. Chem.*, 1964, 29, 1136.

63. A. E. Derome, "Modern N.M.R. Techniques for Chemistry Research," Pergamon, Oxford, 1987, p. 104.

64. H. M. Sirat, E. J. Thomas and N. D. Tyrrell, *J. Chem. Soc. Chem. Commun.*, 1979, 36.

65. T. Mukaiyama and K. Takahashi, *Tetrahedron Lett.*, 1968, 5907.

66. K. S. Boustany and A. B. Sullivan, *Tetrahedron Lett.*, 1970, 3547; D. N. Harpp, D. K. Ash, T. G. Back. J. G. Gleason, B. A. Orwing, W. F. Van Horn and J. P. Snyder, *Tetrahedron Lett.*, 1970, 3551.

67. E. E. Royals and L. L. Harrell Jr., *J. Am. Chem. Soc.*, 1955, 77, 3405.

68. W. D. Emmons and A. S. Pagano, *J. Am. Chem. Soc.*, 1955, 77, 89.

69. E. J. Corey, J. A. Katzenellenbogen, N. W. Gilmann, S. A. Roman and B. W. Erickson, *J. Am. Chem. Soc.*, 1968, 90, 5618.

70. E. S. Gould, R. R. Hiatt and K. C. Irwin, *J. Am. Chem. Soc.*, 1968, 90, 4573; K. B. Sharpless and T. R. Verhoeven, *Aldrichimica Acta*, 1979, 12, 63.

71. G. B. Payne, P. H. Deming and P. H. Williams, *J. Org. Chem.*, 1961, 26, 659; G. B. Payne, *Tetrahedron*, 1962, 18, 763.

72. See for example, R. B. Woodward, J. Gosteli, I. Ernest, R. J. Friary, G Nestller, H. Raman, R. Sitrin, Ch. Suter and J. K. Whitesell, *J. Am. Chem. Soc.*, 1973, 95, 6853.

73. C. H. Gagnieu and A. V. Grouiller, *J. Chem. Soc., Perkin Trans I.*, 1982, 1009.

74. G. Berti, *Topics in Stereochem.*, 1973, 7, 93.

75. E. Weitz and A. Scheffer, *Chem. Ber.*, 1921, 54, 2327.

76. V. G. Dryuk, *Tetrahedron*, 1976, 32, 2855.

77. N. S. Crossley, A. C. Darby, H. B. Henbest, J. J. McCullough, B. Nicholls and M. F. Stewart, *Tetrahedron Lett.*, 1961, 398; H. B. Henbest, *Proc. Chem. Soc.*, 1958, 225.

78. H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, 1957, 1958.

79. I. G. Young, F. Gibson and C. G. MacDonald, *Biochim. Biophys. Acta.*, 1969, 192, 62.

80. B. A. Chiasson and G. A. Berchtold, *J. Am. Chem. Soc.*, 1974, **96**, 2898.

81. I. G. Young, F. Gibson, *Biochim. Biophys. Acta.*, 1969, **177**, 182.

82. L. M Jackman and S. Sternhill, "Applications of NMR Spectroscopy in Organic Chemistry", Pergamon Press, London, 1969, second edn.

83. J. B. Lambert and D. E. Marko, *J. Am. Chem. Soc.*, 1985, **107**, 7978.

84. P. Bakuzis and M. L. F. Bakuzis, *J. Org. Chem.*, 1981, **46**, 235.

85. P. Sykes, "A Guidebook to Mechanism in Organic Chemistry," Longman, London, 1981, fifth edn., p. 65.

86. F. G. Bordwell and H. M. Andersen, *J. Am. Chem. Soc.*, 1953, **75**, 6019.

87. T. W. Greene, "Protective Groups in Organic Synthesis," Wiley, Chichester, 1981, and references therein.

88. M. E. Jung and M. A. Lyster, *J. Am. Chem. Soc.*, 1977, **99**, 968.

89. T. Morita, Y. Okamoto and H. Sakurai, *J. Chem. Soc., Chem. Commun.*, 1978, 874; G. A. Olah, S. C. Narang, B. G. Balaram Gupta and R. Malhotra, *J. Org. Chem.*, 1979, **44**, 1247.

90 S. Kobayashi, K. Kamiyama, T. Iimori and M. Ohno, *Tetrahedron Lett.*, 1984, 2557.

91. M. E. Jung, W. A. Andrus and P L. Omstein, *Tetrahedron Lett.*, 1977, 4195; M. E. Jung and M. A. Lyster, *J. Org. Chem.*, 1977, **42**, 3761.

92. J. B. Jones, *Tetrahedron*, 1986, **42**, 3351.

93. A. J. Adler and G. B. Kistiakowsky, *J. Biol. Chem.*, 1961, **236**, 3240; A. J. Adler and G. B. Kistiakowsky, *J. Am. Chem. Soc.*, 1962, **84**, 695; P. R. Ocken and M. Levy, *Biochim. Biophys. Acta.*, 1970, **212**, 450.

94. (a) E. Heymann and W. Junge, *Eur. J. Biochem.*, 1979, 95, 509; (b) E. Heymann and W. Junge, *Eur. J. Biochem.*, 1979, 95, 519;

95. (a) D. Farb and W. P. Jencks, *Arch. Biochem. Biophys.*, 1980, 203, 214; (b) D. Farb and W. P. Jencks, *Arch. Biochem. Biophys.*, 1980, 203, 227.

96. P. Greenzaid and W. P. Jencks, *Biochemistry*, 1971, 10, 1210.

97. F. Björkling, T. Boutelje, S. Gatenbeck, K. Hult, T. Norin and P. Szmulik, *Tetrahedron*, 1985, 41, 1347.

98. A. Fischili, in "Modern Synthetic Methods," ed. R. Scheffold, Salle/Sauerländer, Frankfurt, 1980, vol. 2, p. 269.

99. Y-Fong Wang, C-Shih Chen, G. Girdaukas and C. J. Sih, in "Enzymes in Organic Synthesis, "(Ciba Foundation Symposium III), Pitman, London, 1985, pp. 128-145; W. E. Ladner and G. M. Whitesides, *J. Am. Chem. Soc.*, 1984, 106, 7250.

100. S. Sicsic, J. Leroy and C. Wakselman, *Synthesis*, 1987, 155.

101. A. J. Shuker, Final Year Research Project, Bath, 1988.

102. A. I. Vogel, "A Text-book for Practical Organic Chemistry," Longman, London, fourth edn., 1978, pp. 289-291.

103. P-T. Ho, *Tetrahedron Lett.*, 1978, 1623.

104. T. H. Black, *Aldrichimica Acta*, 1983, 16, 3.

103. R. Grewe and A. Bokranz, *Chem Ber.*, 1955, 91, 2452.

106. M. Schlosser, *Tetrahedron*, 1978, 34, 3.

107. See for example : J. G. Buchanan and H. Z. Sable, in "Selective Organic Transformations," ed. B. S. Thyagarajan, Wiley, New York, 1972, vol. 2, pp. 1-95; J. Gorzynski Smith, *Synthesis*, 1984, 629; A. S. Rao, S. K. Paknikar and J. G. Kirtane, *Tetrahedron*, 1983, 39, 2323.

108. G. A. Olah, J. T. Welch, Y. D. Vankar, M. Nojima, I. Kerekes and J. A. Olah, *J. Org. Chem.*, 1979, 44, 3872; A. Ourari, R. Condom and R. Guedj, *Can. J. Chem.*, 1982, 60, 2707.

109. J. W. Elmsley, L. Phillips and V. Wray, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1976, 10, pp. 83-756.

110. See for example, F. Camps, G. Fabrias and A. Guerrero, *Tetrahedron*, 1986, (42), 3623.

111. E. Pretsch, J. T. Clerc, J. Seibl and W. Simon, "Tables of Spectral Data for Structure Determination of Organic Compounds," eds. F. L. Boschke, W. Fresenius, J. F. K. Huber, E. Pungor, G. A. Rechnitz, W. Simon and Th. S. West, Springer-Verlag, Berlin Heidelberg, 1981, English Translation of the revised second German edn., p. C240.

112. P. G. Gassman and T. L. Guggehneim, *J. Am. Chem. Soc.*, 1982, 104, 5849; P. G. Gassman and R. S. Gremban, *Tetrahedron Lett.*, 1984, 3259.

113. W. Lidy and W. Sundermeyer, *Tetrahedron Lett.*, 1973, 1449.

114. S. F. Dyke, A. J. Floyd, M. Sainsbury and R. S. Theobald, "Organic Spectroscopy : An Introduction", Longman, London, 1978, second edn., p. 86.

115. E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, 1972, 94, 6190.

116. E. J. Corey, H. Cho, C. Rücker and D. H. Hua, *Tetrahedron Lett.*, 1981, 3455.

117. See for example : P. Chamberlain, M. L. Roberts and G. H. Whitham, *J. Chem. Soc. B.*, 1970, 1374.

118. D. A. McGowan and G. A. Berchtold, *J. Am. Chem. Soc.*, 1982, 104, 7036.

119. T. Fex, J. Trofast and B. Wickberg, *Acta Chem. Scand. B35*, 1981, 97.

120. R. M. Silverstein, G. C. Bassler and T. C. Morrill, "Spectrometric Identification of Organic Compounds," Wiley, London, 1974, third edn., p. 169.

121. D. Sinou and M. Emziane, *Synthesis*, 1986, 1045.

122. D. D. Perrin, W. L. F. Armarego and D. R. Perrin, "Purification of Laboratory Chemicals", Pergamon, Oxford, 1980, Second edn.

123. L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Wiley, New York, 1967.

124. R. Daniels and J. L. Fischer, *J. Org. Chem.* 1963, 28, 320.

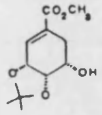
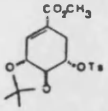
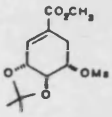
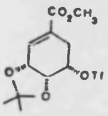
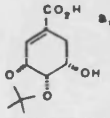
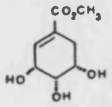
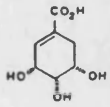
125. R. Grewe and S. Kersten, *Chem. Ber.*, 1967, 100, 2546.

126. R. I. Christopherson and J. F. Morrison, *Arch. Biochem. and Biophys.*, 1983, 220, 444.

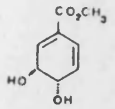
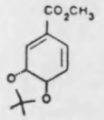
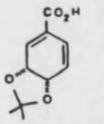
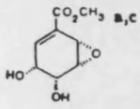
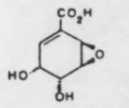
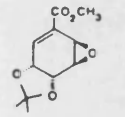
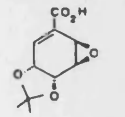
APPENDICES

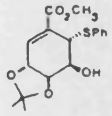
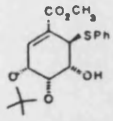
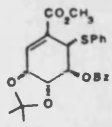
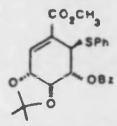
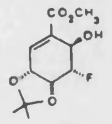
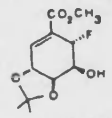
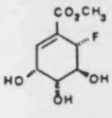
APPENDIX I

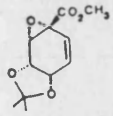
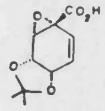
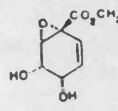
Tables of ^{1}H n.m.r. Data

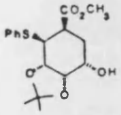
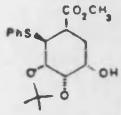
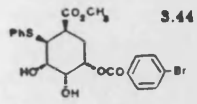
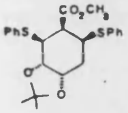
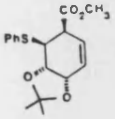
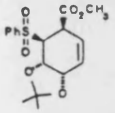
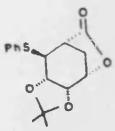
COMPOUND	2-H	3-H	4-H	5-H	6α-H	6β-H	CO_2CH_3	OTHERS
	6.78 $J_{2,3}$=3.5 $J_{2,6\alpha}$=2.5 $J_{2,6\beta}$=1.0	4.74 $J_{3,4}$=6.5 $J_{3,2}$=3.5 $J_{3,6\alpha}$=2.5	4.42 $J_{4,3}$=6.5 $J_{4,5}$=3.0	3.95	2.48 J_{gem}=19.0 $J_{6\alpha,5}$=10.5 $J_{6\alpha,2}$=2.5 $J_{6\alpha,3}$=2.5	2.65 J_{gem}=10.5 $J_{6\beta,5}$=6.0	3.79	1.41&1.51 (CMe_2) 1.98 (OH)
	6.72	4.70	4.40	4.77	2.60	2.60	3.76	1.34(CMe_2) 2.46(Ar-Me) 7.36 & 7.86 (Ar)
	6.77 $J_{2,3}$=2.5 $J_{2,6\alpha}$=2.5 $J_{2,6\beta}$=1.0	4.81	4.57 $J_{4,3}$=5.0 $J_{4,5}$=2.0 $J_{4,6\beta}$=1.0	4.94 $J_{5,6\alpha}$=10.0 $J_{5,6\beta}$=5.5 $J_{5,4}$=2.5	2.80 J_{gem}=16.5 $J_{6\alpha,5}$=10.0 $J_{6\alpha,2}$=2.5 $J_{6\alpha,3}$=2.5	2.87 J_{gem}=16.5 $J_{6\beta,5}$=5.5 $J_{6\beta,2}$=1.0	3.79	1.39 & 1.42 (CMe_2) 3.13 (SO_2CH_3)
	6.83	4.83 $J_{3,4}$=5.5 $J_{3,2}$=3.5 $J_{3,6\alpha}$=2.0	4.53 $J_{4,3}$=5.5 $J_{4,5}$=2.5	5.11 $J_{5,6\alpha}$=9.0 $J_{5,6\beta}$=5.5 $J_{5,4}$=2.5	2.91 J_{gem}=16.5 $J_{6\alpha,5}$=9.0 $J_{6\alpha,2}$=2.0 $J_{6\alpha,3}$=2.0	2.84 $J_{6\beta,5}$=5.5	3.80	1.41&1.43 (CMe_2)
 a,b	6.65 $J_{2,3}$=3.0 $J_{2,6\alpha}$=3.0 $J_{2,4}$=1.0	4.75 $J_{3,6\alpha}$=3.0 $J_{3,6\beta}$=1.0	4.41 $J_{4,3}$=5.0 $J_{4,5}$=2.5 $J_{4,2}$=1.0	3.90 $J_{5,6\alpha}$=10.0 $J_{5,6\beta}$=5.5 $J_{5,4}$=2.5	2.34 J_{gem}=16.5 $J_{6\alpha,5}$=10.0 $J_{6\alpha,3}$=3.0	2.60 J_{gem}=16.5 $J_{6\beta,5}$=5.5 $J_{6\beta,3}$=1.0		1.32 & 1.37 (CMe_2)
 c	6.65 $J_{2,6\alpha}$=2.5 $J_{2,3}$=1.5	4.29	4.01 $J_{4,3}$=2.5 $J_{4,5}$=1.0	3.84 $J_{5,6\alpha}$=9.0 $J_{5,6\beta}$=5.5 $J_{5,4}$=1.0	2.37 J_{gem}=17.0 $J_{6\alpha,5}$=9.0 $J_{6\alpha,3}$=3.0 $J_{6\alpha,2}$=2.5	2.60 J_{gem}=17.0 $J_{6\beta,5}$=5.5 $J_{6\beta,3}$=1.0	3.71	2.89 & 3.95 (OH)
	6.65 & 6.69	4.29	3.94 $J_{4,3}$=3.5 $J_{4,5}$=2.0	3.85 $J_{5,6\alpha}$=9.0 $J_{5,6\beta}$=6.0 $J_{5,3}$=2.0 $J_{5,4}$=2.0	2.38 J_{gem}=17.0 $J_{6\alpha,5}$=9.0 $J_{6\alpha,3}$=3.0 $J_{6\alpha,2}$=2.5	2.50 J_{gem}=17.0 $J_{6\beta,5}$=6.0 $J_{6\beta,3}$=2.0		3.20 (3OH)

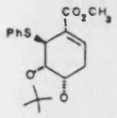
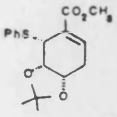
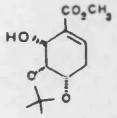
a D_2O added; b Spectrum recorded in CD_3OD;c Spectrum recorded in $(CD_3)_2CO$;
d Spectrum recorded in C_6D_6.

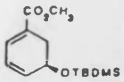
COMPOUND	2-H	3-H	4-H	5-H	6-H	CO_2CH_3	OTHERS
 c	6.88	4.40 $J_{3,4}$=6.5 $J_{3,2}$=3.5	4.15	6.11 $J_{5,6}$=10.0 $J_{5,4}$=5.0	6.39 $J_{6,5}$=10.0	3.77	3.80 (OH) 4.12 (OH)
	6.86 $J_{2,3}$=4.0 $J_{2,6}$=1.5 $J_{2,5}$=1.0	4.81 $J_{3,4}$=9.0 $J_{3,2}$=4.0	4.65 $J_{4,3}$=9.0 $J_{4,5}$=4.0 $J_{4,6}$=1.0	6.04 $J_{5,6}$=10.0 $J_{5,4}$=4.0 $J_{5,2}$=1.0	6.54 $J_{6,5}$=10.0	3.80	1.39 & 1.41(CMe_2)
	7.00 $J_{2,3}$=3.5 $J_{2,6}$=1.0	4.85 $J_{3,4}$=9.0 $J_{3,2}$=3.5	4.66 $J_{4,3}$=9.0 $J_{4,5}$=4.0	6.07 $J_{5,6}$=10.0 $J_{5,4}$=4.0	6.54 $J_{6,5}$=10.0	6.88	1.41 and 1.43(CMe)
 a,c	7.17 $J_{2,3}$=4.0 $J_{2,6}$=1.5	4.32 $J_{3,2}$=4.0 $J_{3,4}$=3.0 $J_{3,5}$=1.5	4.09 $J_{4,3}$=3.0	3.74 $J_{5,6}$=2.5 $J_{5,4}$=1.5	4.00 $J_{6,5}$=2.5 $J_{6,2}$=1.5	3.83	
 a	6.82 $J_{2,3}$=4.0	4.42 $J_{3,2}$=4.0 $J_{3,4}$=4.0	3.76 $J_{4,5}$=8.0 $J_{4,3}$=4.0	4.00 $J_{5,4}$=8.0 $J_{5,6}$=5.0	4.34 $J_{6,5}$=5.0		
	6.81 $J_{2,3}$=7.0 $J_{2,6}$=1.5 $J_{2,4}$=0.5	4.58 $J_{3,4}$=7.0 $J_{3,2}$=2.5	4.81 $J_{4,3}$=7.0 $J_{4,5}$=2.0 $J_{4,2}$=0.5 $J_{4,6}$=0.5	3.67 $J_{5,6}$=3.5 $J_{5,4}$=2.0	4.00 $J_{6,5}$=3.5 $J_{6,2}$=1.5 $J_{6,4}$=0.5	3.83	1.37 & 1.41(CMe_2)
	6.98 $J_{2,3}$=2.5 $J_{2,6}$=1.5	4.62 $J_{3,4}$=7.0 $J_{3,2}$=2.5	4.83 $J_{4,3}$=7.0 $J_{4,5}$=2.5	3.70 $J_{5,6}$=3.5 $J_{5,4}$=2.5	4.01 $J_{6,5}$=3.5 $J_{6,2}$=1.5	8.70	

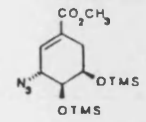
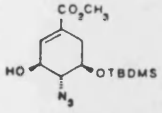
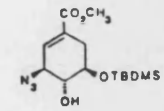
COMPOUND	2-H	3-H	4-H	5-H	6-H	CO_2CH_3	OTHERS
	6.90 $J_{2,3}$=3.5	4.60 $J_{3,4}$=7.0 $J_{3,2}$=3.5	4.29 $J_{4,5}$=9.0 $J_{4,3}$=7.0	3.90 $J_{5,4}$=9.0 $J_{5,OH}$=7.0 $J_{5,6}$=4.5	4.40 $J_{6,5}$=4.5	3.74	1.37 & 1.48(CMe_2) 2.45 (OH) 7.30 & 7.55(SPh)
	6.78 $J_{2,3}$=4.0	4.74 $J_{3,4}$=5.0 $J_{3,2}$=4.0 $J_{3,6}$=1.0	4.37 $J_{4,3}$=5.0 $J_{4,5}$=5.0	4.58	4.02 $J_{6,5}$=2.5 $J_{6,3}$=1.0	3.73	1.41 & 1.47(CMe_2) 2.26 (OH) 7.29 & 7.60(SPh)
 d	6.96	4.42 $J_{3,4}$=7.0 $J_{3,2}$=3.5	5.00 $J_{4,5}$=9.0 $J_{4,3}$=7.0	5.56 $J_{5,4}$=9.0 $J_{5,6}$=4.0	5.18 $J_{6,5}$=4.0	3.34	1.20 & 1.40(CMe_2) 6.68,7.02,7.56 & 7.89 (SPh & OCOPh)
	6.85 $J_{2,3}$=3.5	4.86 $J_{3,4}$=5.0 $J_{3,2}$=3.5 $J_{3,6}$=1.0	4.53	6.11 $J_{5,4}$=3.5 $J_{5,6}$=2.0	4.22 $J_{6,5}$=2.5 $J_{6,3}$=1.0	3.63	1.44 & 1.54(CMe_2) 7.22-7.62 & 7.93 (SPh & OCOPh)
	6.94 $J_{2,3}$=3.5 $J_{2,4}$=2.0 $J_{2,6}$=1.0	4.30 $J_{3,4}$=2.5 $J_{3,2}$=3.0	4.75 $J_{4,5}$=6.5 $J_{4,3}$=2.5 $J_{4,2}$=2.0	4.27 $J_{5,F}$=10.5 $J_{5,4}$=6.5 $J_{5,6}$=5.5	5.25 $J_{6,F}$=46.0 $J_{6,5}$=5.0	3.85	1.26 & 1.49(CMe_2)
	6.83 $J_{2,6}$=2.5 $J_{2,3}$=2.5	4.58 $J_{3,4}$=6.5 $J_{3,2}$=2.0	4.41 $J_{4,F}$=8.0 $J_{4,3}$=6.5 $J_{4,5}$=1.5	5.49 $J_{5,F}$=48.0 $J_{5,6}$=3.0	4.90 $J_{6,F}$=7.0 $J_{6,5}$=3.0 $J_{6,2}$=2.5	3.85	1.44 (CMe_2)
	6.95 $J_{2,3}$=5.0	4.49 $J_{3,2}$=5.0 $J_{3,4}$=4.0	3.69 $J_{4,5}$=9.0 $J_{4,3}$=4.0	4.23 $J_{5,F}$=17.0 $J_{5,4}$=9.0 $J_{5,6}$=6.0	5.23 $J_{6,F}$=48.0 $J_{6,5}$=6.0	3.82	

COMPOUND	2-H	3-H	4-H	5-H	6-H	CO_2CH_3	OTHERS
	3.88 $J_{2,3}$=2.0	4.77 $J_{3,4}$=7.0 $J_{3,2}$=2.0	4.48 $J_{4,3}$=7.0 $J_{4,5}$=2.5 $J_{4,6}$=1.5	5.86 $J_{5,6}$=10.5 $J_{5,4}$=2.5 $J_{5,3}$=0.5	6.40 $J_{6,5}$=10.5 $J_{6,4}$=2.5	3.80	1.38 (CMe_2)
	3.95 $J_{2,3}$=2.0	4.81 $J_{3,4}$=7.0 $J_{3,2}$=2.0	4.51 $J_{4,3}$=7.0 $J_{4,5}$=2.5 $J_{4,6}$=2.0	5.91 $J_{5,6}$=10.0 $J_{5,4}$=2.5	6.38 $J_{6,5}$=10.0 $J_{6,4}$=2.0		1.40(CMe_2)
	3.91 $J_{2,3}$=1.5	4.04 $J_{3,4}$=3.0	4.18 $J_{4,5}$=3.5 $J_{4,3}$=3.0 $J_{4,2}$=1.0	6.27 $J_{5,6}$=7.0 $J_{5,4}$=3.5	6.48 $J_{6,5}$=7.0	3.81	

COMPOUND	1-H	2-H	3-H	4-H	5-H	6α-H	6β-H	CO_2CH_3	OTHERS
	3.26 $J_{1,6\beta}$=6.5 $J_{1,6\alpha}$=6.5 $J_{1,2}$=4.5	3.48 $J_{2,3}$=6.0 $J_{2,1}$=4.5	4.55 $J_{3,2}$=6.0 $J_{3,4}$=6.0	4.43 $J_{4,3}$=6.0 $J_{4,5}$=3.5	4.15	1.95 J_{gem}=14.0 $J_{6\alpha,5}$=9.0 $J_{6\alpha,1}$=6.5	2.17 J_{gem}=14.0 $J_{6\beta,1}$=6.5 $J_{6\beta,5}$=6.0	3.71	1.39,1.52(CMe_2) 2.27(OH) 7.24-7.34,7.4 (SPh)
	2.46	3.21 $J_{2,1}$=11.5 $J_{2,3}$=9.0	4.01 $J_{3,2}$=9.0 $J_{3,4}$=5.0	4.29 $J_{4,3}$=5.0 $J_{4,5}$=4.0	3.82	2.46	2.46	3.69	1.38,1.51(CMe_2) 2.00(OH) 7.28-7.33,7.5 (SPh)
	3.44	3.71	4.28 $J_{3,2}$=6.0 $J_{3,4}$=3.0	4.40 $J_{4,5}$=3.5 $J_{4,3}$=3.0	5.44 $J_{5,6\alpha}$=7.0 $J_{5,4}$=3.5 $J_{5,6\alpha}$=3.5	2.33 J_{gem}=14.0 $J_{6\alpha,5}$=7.0 $J_{6\alpha,1}$=4.5	2.15 J_{gem}=14.0 $J_{6\beta,1}$=8.0 $J_{6\beta,5}$=3.5	3.71	2.05(2OH) 7.25-7.60,7.9 (SPh,OCOAr)
					5α-H	5β-H	6-H		
	3.16 $J_{1,2}$=4.5 $J_{1,6}$=4.0	3.00 $J_{2,3}$=9.5 $J_{2,1}$=4.5	4.40 $J_{3,2}$=9.5 $J_{3,4}$=5.0	4.43 $J_{4,5\beta}$=3.5	1.77	1.77	3.42 $J_{6,5\beta}$=10.5 $J_{6,5\alpha}$=6.5 $J_{6,1}$=4.0	3.79	1.3,1.49(CMe_2) 7.26,7.39,7.46 (SPh)
					5-H	6-H			
	3.72	3.81 $J_{2,1}$=5.0 $J_{2,3}$=5.5	4.62 $J_{3,2}$=5.5 $J_{3,4}$=5.5	4.76	5.91 $J_{5,6}$=10.0 $J_{5,4}$=3.0 $J_{5,1}$=2.5	6.12 $J_{6,5}$=10.0 $J_{6,1}$=3.0		3.72	1.38,1.39(CMe_2) 6.92,7.41(SPh)
	3.87	3.55 $J_{2,3}$=9.0 $J_{2,1}$=4.5	5.18 $J_{3,2}$=8.5 $J_{3,4}$=6.5	4.76	6.01 $J_{5,6}$=9.5 $J_{5,4}$=3.5 $J_{5,1}$=1.5	6.12 $J_{6,5}$=9.5 $J_{6,1}$=5.5 $J_{6,4}$=1.0		3.79	1.06,1.31$(CMe)_2$
					5-H	8-*endo*-H	8-*exo*-H		
	2.63	3.98 $J_{2,1}$=2.5 $J_{2,3}$=1.0	4.40 $J_{3,4}$=6.0	4.21 $J_{4,3}$=6.0 $J_{4,5}$=2.5	4.67 $J_{5,8exo}$=5.5 $J_{5,4}$=2.5 $J_{5,8exo}$=1.0 $J_{5,8endo}$=1.0	2.30 J_{gem}=13.0 $J_{8,5}$=1.0 $J_{8,1}$=2.0	2.38 J_{gem}=13.0 $J_{8,5}$=5.5		1.30,1.54(CMe_2)

COMPOUND	2-H	3-H	4-H	5α-H	5β-H	6-H	CO_2CH_3	OTHERS
	4.36 $J_{2,3}$=3.5 $J_{2,5\alpha}$=3.5	4.65 $J_{3,4}$=6.5 $J_{3,2}$=3.5	4.84 $J_{4,5\alpha}$=7.0 $J_{4,3}$=6.5 $J_{4,5\beta}$=5.5	2.20 J_{gem}=13.5 $J_{5\alpha,4}$=7.0 $J_{5\alpha,2}$=3.5	2.27	7.01 $J_{6,5\beta}$=3.5	3.77	1.26 & 1.28(CMe_2) 7.25-7.60(SPh)
	4.52 $J_{2,3}$=1.5	4.62 $J_{3,4}$=7.0 $J_{3,2}$=1.5	4.65	2.57 J_{gem}=18.0 $J_{5\alpha,4}$=7.0 $J_{5\alpha,6}$=2.0	2.50 J_{gem}=18.0 $J_{5\beta,4}$=4.0 $J_{5\beta,6}$=3.5	7.17 $J_{6,5\beta}$=3.5 $J_{6,5\alpha}$=2.0	3.78	1.27 & 1.30(CMe_2) 7.20-7.25(SPh)
	4.71 $J_{2,3}$=2.5	4.41 $J_{3,4}$=6.5 $J_{3,2}$=2.5	4.51 $J_{4,3}$=6.5 $J_{4,5\beta}$=5.0 $J_{4,5\alpha}$=3.0	2.55 J_{gem}=18.0 $J_{5\alpha,6}$=5.5 $J_{5\alpha,4}$=3.0	2.66 J_{gem}=18.0 $J_{5\beta,4}$=5.0 $J_{5\beta,6}$=3.5	7.10 $J_{6,5\alpha}$=5.5 $J_{6,5\beta}$=3.5	3.79	1.35 & 1.36(CMe_2) 2.67(OH)

COMPOUND	2-H	3-H	4-H	5-H	6α-H	6β-H	CO_2CH_3	OTHERS
	7.04	6.16	6.16	4.35	2.67 J_{gem}=16.0 $J_{6\alpha,5}$=8.0	2.76 J_{gem}=16.0 $J_{6\beta,5}$=12.0	3.73	3.25 (OH)
	7.00	6.07	6.07	4.50 $J_{5,6\beta}$=10.0 $J_{5,6\alpha}$=7.5 $J_{5,4}$=2.0	2.63	2.63	3.77	0.20($SiMe_2$) 1.00(*t*-Bu)
	7.02 $J_{2,3}$=4.0 $J_{2,6\beta}$=3.5	3.53 $J_{3,4}$=4.5 $J_{3,2}$=4.0	3.68	4.15 $J_{5,6\beta}$=10.0 $J_{5,6\alpha}$=6.5 $J_{5,4}$=1.0	2.90 J_{gem}=16.5 $J_{6\alpha,5}$=6.5 $J_{6\alpha,4}$=2.0	2.14 J_{gem}=16.5 $J_{6\beta,5}$=10.0 $J_{6\beta,2}$=3.5	3.76	3.77(OH)
	7.14 $J_{2,3}$=4.0 $J_{2,6\alpha}$=3.5	3.50 $J_{3,2}$=4.0 $J_{3,4}$=3.5	3.61	4.58 $J_{5,6\alpha}$=5.0 $J_{5,6\beta}$=2.5 $J_{5,4}$=2.0	2.30 J_{gem}=17.5 $J_{6\alpha,5}$=5.0 $J_{6\alpha,2}$=3.5	2.80 J_{gem}=17.5 $J_{6\beta,5}$=2.5 $J_{6\beta,4}$=2.0	3.77	3.77(OH)
	7.00 $J_{2,3}$=4.0 $J_{2,6}$=4.0	3.46 $J_{3,2}$=4.0 $J_{3,4}$=1.0	3.51	4.14 $J_{5,6\beta}$=10.0 $J_{5,6\alpha}$=6.5 $J_{5,4}$=1.0	2.73 J_{gem}=16.5 $J_{6\alpha,5}$=6.5 $J_{6\alpha,4}$=2.0	2.21 J_{gem}=16.5 $J_{6\beta,5}$=10.0 $J_{6\beta,2}$=3.5	3.75	0.19($SiMe_3$)
	7.09 $J_{2,3}$=4.0 $J_{2,6\alpha}$=3.0	3.44 $J_{3,4}$=4.5 $J_{3,2}$=4.0	3.40 $J_{4,3}$=4.5 $J_{4,5}$=2.5 $J_{5,6\beta}$=2.0	4.49 $J_{5,6\alpha}$=5.0 $J_{5,4}$=2.5 $J_{5,6\beta}$=2.0	2.22 J_{gem}=17.5 $J_{6\alpha,5}$=5.0 $J_{6\alpha,2}$=3.0	2.65 J_{gem}=17.5 $J_{6\beta,5}$=2.0 $J_{6\beta,4}$=2.0	3.73	0.12 ($SiMe_3$)
	7.00 $J_{2,3}$=3.5 $J_{3,6\beta}$=3.5	3.46 $J_{3,4}$=4.0 $J_{3,2}$=3.5	3.51 $J_{4,3}$=4.0 $J_{4,6\alpha}$=2.0 $J_{4,5}$=1.0	4.15 $J_{5,6\beta}$=10.0 $J_{5,6\alpha}$=6.5 $J_{5,4}$=1.0	2.72 J_{gem}=16.5 $J_{6\alpha,5}$=6.5 $J_{6\alpha,4}$=2.0	2.22 J_{gem}=16.5 $J_{6\beta,5}$=10.0 $J_{6\beta,2}$=3.5	3.75	0.05 & 0.08($SiMe_2$) 0.93 (Si*t*-Bu)
	7.08 $J_{2,3}$=4.0 $J_{3,6\alpha}$=3.0	3.45 $J_{3,2}$=4.0 $J_{3,4}$=4.0	3.41 $J_{4,3}$=4.0 $J_{4,5}$=2.5 $J_{4,6\beta}$=2.0	4.51 $J_{5,6\alpha}$=5.0 $J_{5,4}$=2.5 $J_{5,6\beta}$=2.0	2.21 J_{gem}=17.0 $J_{6\alpha,5}$=5.0 $J_{6\alpha,2}$=3.0	2.66 J_{gem}=17.0 $J_{6\beta,5}$=2.0 $J_{6\beta,4}$=2.0	3.74	0.07 & 0.09($SiMe_2$) 0.85 (Si*t*-Bu)

COMPOUND	2-H	3-H	4-H	5-H	6α-H	6β-H	CO_2Me	OTHERS
	6.92	4.72 $J_{3,2}$=2.5 $J_{3,4}$=2.0	4.10 $J_{4,3}$=2.0 $J_{4,5}$=2.0	4.43 $J_{5,6\beta}$=9.0 $J_{5,6\alpha}$=6.0 $J_{5,4}$=2.0	2.64 J_{gem}=18.0 $J_{6\alpha,5}$=6.0	2.75 J_{gem}=18.0 $J_{6\beta,5}$=9.0 $J_{6\beta,2}$=2.0	3.75	0.13 ($2SiMe_3$)
	7.02 $J_{2,3}$=4.0 $J_{2,6\beta}$=3.0	4.78 $J_{3,4}$=8.5 $J_{3,2}$=4.0 $J_{3,6\beta}$=4.0	3.94 $J_{4,5}$=9.5 $J_{4,3}$=8.5 $J_{4,6\alpha}$=1.5	3.58 $J_{5,6\beta}$=10.0 $J_{5,4}$=9.5 $J_{5,6\alpha}$=5.5	2.78 J_{gem}=17.5 $J_{6\alpha,5}$=5.5 $J_{6\alpha,4}$=1.5	2.32 J_{gem}=17.5 $J_{6\beta,5}$=9.5 $J_{6\beta,3}$=4.0 $J_{6\beta,2}$=3.0	3.77	0.13&0.14($SiMe_2$) 0.91(Si*t*-Bu) 2.73(OH)
	7.05 $J_{2,3}$=5.0 $J_{2,6\beta}$=2.5	5.13 $J_{3,2}$=5.0 $J_{3,4}$=4.0	2.71 $J_{4,5}$=9.0 $J_{4,3}$=4.0 $J_{4,6\beta}$=1.5	4.09 $J_{5,6\beta}$=9.0 $J_{5,4}$=9.0 $J_{5,6\alpha}$=6.0	2.98 J_{gem}=17.5 $J_{6\alpha,5}$=6.0	2.55 J_{gem}=17.5 $J_{6\beta,5}$=9.0 $J_{6\beta,2}$=2.5 $J_{6\beta,4}$=1.5	3.76	0.15&0.17 ($SiMe_2$) 0.93(Si*t*-Bu) 2.82(OH)

APPENDIX II

Table of Bond Lengths and Bond Angles for (110)

Br1	C1		1.857	C7	C4		1.480	C11	C10		1.586
				C7	O1		1.262	C11	C12		1.610
C1	Br1		1.857	C4	C7	O1	121.2	C10	C11	C12	110.7
C1	C2		1.395	C7	O2		1.311	C11	S1		1.864
Br1	C1	C2	121.0	C4	C7	O2	116.6	C10	C11	S1	108.7
C1	C6		1.395	O1	C7	O2	122.1	C12	C11	S1	107.3
Br1	C1	C6	118.92								
C2	C1	C6	120.0	O1	C7		1.262	C12	C11		1.610
								C12	C13		1.578
C2	C1		1.395	O2	C7		1.311	C11	C12	C13	109.0
C2	C3		1.395	O2	C8		1.425	C12	O6		1.469
C1	C2	C3	120.0	C7	O2	C8	119.8	C11	C12	O6	97.9
								C13	C12	O6	107.0
C3	C2		1.395	C8	O2		1.425				
C3	C4		1.395	C8	C9		1.589	C13	C8		1.432
C2	C3	C4	120.0	O2	C8	C9	106.2	C13	C12		1.578
				C8	C13		1.432	C8	C13	C12	122.9
C4	C3		1.395	O2	C8	C13	109.3	C13	O5		1.429
C4	C5		1.395	C9	C8	C13	112.8	C8	C13	O5	114.4
C3	C4	C5	120.0					C12	C13	O5	102.5
C4	C7		1.480	C9	C8		1.589				
C3	C4	C7	117.8	C9	C10		1.476	C14	C10		1.530
C5	C4	C7	122.2	C8	C9	C10	108.3	C14	O3		1.263
								C10	C14	O3	123.0
C5	C4		1.395	C10	C9		1.476	C14	O4		1.312
C5	C6		1.395	C10	C11		1.586	C10	C14	O4	114.4
C4	C5	C6	120.0	C9	C10	C11	114.6	O3	C14	O4	122.5
				C10	C14		1.530				
C6	C1		1.395	C9	C10	C14	113.0	O3	C14		1.263
C6	C5		1.395	C11	C10	C14	108.2				
C1	C6	C5	120.0								

O4	C14		1.312	C19	C18		1.395
O4	C15		1.456	C19	C20		1.395
C14	O4	C15	118.4	C18	C19	C20	120.0
C14	O4		1.456	C20	C19		1.395
				C20	C21		1.395
O5	C13		1.429	C19	C20	C21	120.0
O6	C12		1.469	C21	C16		1.395
				C21	C20		1.395
S1	C11		1.864	C16	C21	C20	120.0
S1	C16		1.693				
C11	S1	C16	100.3				
C16	S1		1.693				
C16	C17		1.395				
S1	C16	C17	121.3				
C16	C21		1.395				
S1	C16	C21	118.5				
C17	C16	C21	1.395				
C17	C16		1.395				
C17	C18		1.395				
C16	C17	C18	120.0				
C18	C17		1.395				
C18	C19		1.395				
C17	C18	C19	120.0				

APPENDIX III

Table of Bond Lengths and Bond Angles for (112)

S1	C4		1.816	C3	03		1.434	C8	S1		1.774
S1	C3		1.774	C3	C2		1.549	C8	C9		1.375
C4	S1	C8	103.9	C2	C3	03	102.1	C9	C8	S1	126.2
				C3	C4		1.534	C8	C13		1.423
O1	C1		1.472	C4	C3	03	108.1	C13	C8	S1	114.0
O1	C7		1.371	C2	C3	C4	116.6	C13	C8	C9	119.7
C1	O1	C7	107.7								
				C4	S1		1.816	C9	C8		1.375
O2	C7		1.185	C4	C3		1.539	C9	C10		1.395
				C3	C4	S1	111.9	C10	C9	C8	120.4
O3	C3		1.434	C4	C5		1.553				
O4	C14		1.450	C5	C4	S1	105.9	C10	C9		1.395
C3	O3	C14	104.6	C3	C4	C5	110.9	C10	C11		1.366
								C9	C10	C11	118.2
O4	C2		1.411	C5	C4		1.553				
O4	C14		1.419	C5	C6		1.537	C11	C10		1.366
C2	O4	C14	119.9	C4	C5	C6	109.9	C11	C12		1.366
				C5	C7		1.512	C10	C11	C12	122.6
C1	O1		1.472	C7	C5	C4	108.1				
C1	C2		1.514	C7	C5	C6	102.2	C12	C11		1.329
O1	C1		110.1					C12	C13		1.380
C1	C6		1.514	C6	C1		1.514	C11	C12	C13	121.4
O1	C1	C6	103.9	C6	C5		1.537				
C2	C1	C6	111.0	C1	C6	C5	97.8	C13	C8		1.423
								C13	C12		1.380
C2	O4		1.411	C7	O1		1.371	C8	C12	C13	117.7
C2	C1		1.514	C7	O2		1.185				
C1	C2	O4	112.7	O1	C7	O2	121.8				
C2	C3		1.549	C7	C5		1.512				
C3	C2	04	102.7	C5	C7	O1	108.4				
C1	C2	C3	113.1	C5	C7	02	129.8				

C14	O3		1.450
C14	O4		1.419
O3	C14	O4	105.2
C14	C15		1.506
C15	C14	O3	110.2
C15	C14	O4	109.9
C14	C16		1.496
C16	C14	O3	107.7
C16	C14	O4	111.9
C16	C14	C15	111.7

C15	C14	1.506

C16	C14	1.496

APPENDIX IV

Biological Testing

1. *E. coli* N99 was grown on M9 minimal salts agar inocculated by flooding. Susceptibility discs were loaded with 50μg of drug, and three discs per agar plate were loaded. Each plate had a disc of sulphamethoxazole for comparison. The plates were incubated at 37°C overnight, and active compounds (*ie* those that inhibit growth) give a zone of inhibition which was measured. The results are expressed as radii of zone of inhibition in millimetres. Sulphamethoxazole gave a zone of 17.3mm., but none of our samples gave a zone of inhibition.

A variation of the test is where *E. coli* N99 was grown in M9 minimal salts media in microtitre plates. Drugs were diluted serially from 500μg ml^{-1} to 0.5μg ml^{-1}. Each well contained 5μL of drug and 195μL of cell suspension, and the plates incubated at 37°C. Growth was monitored by measuring absorbance at 620 nm using a Titertek Multiscan. In this test the minimum inhibitory concentration for methyl fluoroshikimate was 125μg ml^{-1}. All other samples showed no inhibition at 500μg ml^{-1}.

2. The supernatant liquor from a sonicated *Klebsilla* mutant (blocked post chorismate) was incubated for 1h. with radiolabelled shikimic acid (1μCu ml^{-1}), a cofactor mixture, and 100μg of "cold" shikimate. This is the control and was compared with the same mixture in the presence of the drug under evaluation. Incubation was carried out under nitrogen since chorismate synthase is sensitive to oxygen. Samples were taken and analysed by t.l.c. using Polygram CEL 300 PEI polyethyleneimine-cellulose (PEI-C) chromatograms[126]. The radioactive products of the reaction were visualised on the plate by autoradiography. The compounds were tested from 50μg ml^{-1} to 1μg ml^{-1}. The effect caused by a putative inhibitor is reflected in the distribution of the radiolabel among the metabolites relative to that control. The results are expressed in total counts in each band and also as a percentage of the equivalent control value (Table IV-1). It can be seen that methyl fluoroshikimate parallels the action of glyphosate which is an inhibitor of EPSP synthetase (as shown by the build up of shikimate-3-phosphate). However the activity of racemic methyl fluoroshikimate is minimal since this level of inhibition requires a concentration of 1μg ml^{-1}.

TABLE IV - 1

Concentration	EPSP	S-3-P	Choris	Shikim	Total	%EPSP	%S-3-P	%Chor	%Shik	% Inhibitation EPSP	% Inhibitation Choris	% Simulation S-3-P	% Simulation Shikim
Control	1696	1286	2073	5226	10281	16.5	12.5	20.2	50.8				
Methyl Fluoroshikimate 1mg ml^{-1}	1090	3794	567	4156	9686	11.3	59.5	5.9	43.3	31.2	70.8	215.8	-14.9
Glyhosate 100 μg ml^{-1}	952	4398	418	4513	10273	9.3	42.7	4.1	43.9	43.8	79.8	241.6	-13.6